A STUDY ON THE ROLE OF FINE NEEDLE ASPIRATION CYTOLOGY OF TESTES IN AZOOSPERMIA

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the regulations

for the award of the degree of

M.Ch. BRANCH - IV

UROLOGY

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI, INDIA

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CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON THE ROLE OF FINE NEEDLE ASPIRATION CYTOLOGY OF TESTES IN AZOOSPERMIA"** is a bonafide work done by **Dr. R.VENKAT KARTHEY** in partial fulfilment of the requirements of The TAMIL NADU DR.M.G.R. MEDICAL UNIVERSITY, Chennai for the award of M.Ch Urology Degree. The period of study was from October 2011 – February 2013

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DECLARATION

I, **Dr. R.VENKAT KARTHEY** solemnly declare that the dissertation titled **"A STUDY ON THE ROLE OF FINE NEEDLE ASPIRATION CYTOLOGY OF TESTES IN AZOOSPERMIA "** is a bonafide work done by me at Govt. Stanley Medical College & Hospital during October 2011 to February 2013 under the guidance and supervision of **Prof .Dr. V. Selvaraj, M.S., M.Ch. (Urology)** Professor and Head Of The Department.

The dissertation is submitted to Tamil Nadu, Dr. M.G.R Medical university, towards partial fulfillment of requirement for the award of M.Ch. Degree(Branch-IV) in urology three years course

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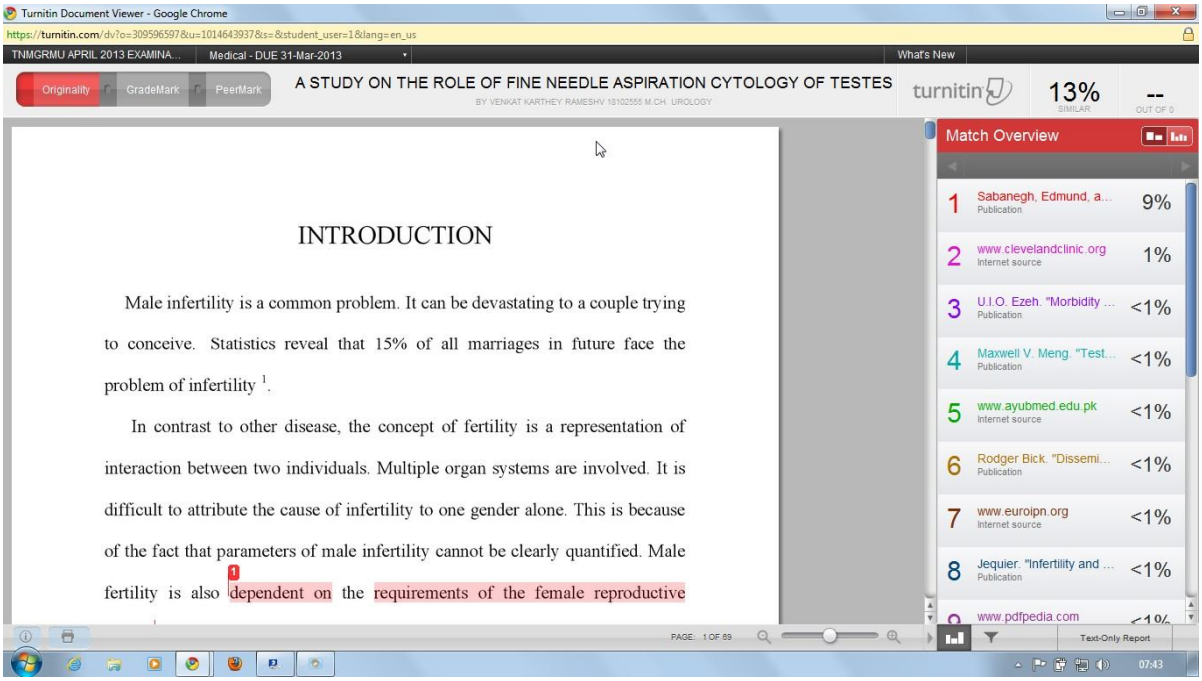
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INTRODUCTION
Male infertility is a common problem. It can be devastating to a couple trying to conceive. Statistics reveal that 15% of all marriages in future face the problem of infertility [1].
In contrast to other disease, the concept of fertility is a representation of interaction between two individuals. Multiple organ systems are involved. It is difficult to attribute the cause of infertility to one gender alone. This is because of the fact that parameters of male infertility cannot be clearly quantified. Male fertility is also dependent on the requirements of the female reproductive
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The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 12.10.2011 at the Modernized Seminar Hall, Stanley Medical College, Chennai-1 at 2PM

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CONTENTS

INTRODUCTION

Male infertility is a common problem. It can be devastating to a couple trying to conceive. Statistics reveal that 15% of all marriages in future face the problem of infertility [1].

In contrast to other disease, the concept of fertility is a representation of interaction between two individuals. Multiple organ systems are involved. It is difficult to attribute the cause of infertility to one gender alone. This is because of the fact that parameters of male infertility cannot be clearly quantified. Male fertility is also dependent on the requirements of the female reproductive system. However data reveal that significant abnormalities in the male alone are responsible for 30 % of cases of infertility. In another 20% of cases, infertility is due to abnormalities in both the male and the female partners. Hence the male factor is at least partially responsible for infertility in approximately 50% of infertile couples .[2]

There have been several advances in our understanding of male infertility and its treatment in the past fifty years. Intracytoplasmic sperm injection (ICSI) was introduced in 1992 [3]. In this technique of in-vitro fertilization a single sperm is directly injected into an egg .As a result of this ability, some of the most severe aetiologies of male sub fertility can be

bypassed. There have also been advances in our understanding of the genetics of fertility and the influence of environmental and endocrine factors on gonadocytes. This has allowed several targeted diagnostic and therapeutic interventions in the field of infertility.

The diagnostic work-up in all patients of male infertility includes detailed clinical evaluation and a standard battery of laboratory investigations including semen analysis, serum hormone assays, immunological studies, etc. Finally, in cases where the semen analysis is abnormal, the status of spermatogenesis in the testis needs to be evaluated by microscopic examination of testicular tissue, conventionally performed by open testicular biopsy. Testicular biopsy can help us to differentiate a post-testicular, obstructive aetiology of male infertility from an intrinsic testicular cause [4]. When post-testicular azoospermia or severe oligospermia is demonstrated, surgical correction may be indicated.

In recent years, several workers have advocated the use of fine needle aspiration cytology as an alternative to biopsy, and have found a good correlation between cytology and histology of the infertile testis. Although first described by Max Huhner, it was not until 1965 when Obrant and Persson did fine needle aspiration (FNA) of human testes in men with fertility disorder that it became popular.[5]

Fine needle aspiration cytology (FNAC) of the testis is a minimally invasive procedure. It is being increasingly used in the evaluation testicular function. Studies have shown a good correlation between FNAC and biopsy findings and abnormal findings in FNAC can be followed up and evaluated further with a formal testicular biopsy. The concordance rate of FNA and histological diagnosis reached >85% in many studies with high specificity and sensitivity approaching >95% [6]. The pathology in the testis may be heterogeneous and hence FNAC may at times provide information not evident on histology. This is because of the ability to sample a wider area of the testis during FNAC. The testis may contain more mature germ cell lineage in small foci far from the site of biopsy.

AIMS AND OBJECTIVES

1) To evaluate cytological features of testicular FNAC in patients with azoospermia

2) To determine the diagnostic values and reliability of testicular FNAC as a cytological sampling technique in azoospermia.

3) Considering histopathology as the ' gold standard ' to study the correlation between cytological and histological diagnosis.

4) To evaluate the possibility of replacing biopsy of azoospermic testes by FNAC for diagnostic purpose.

5) To study the need for bilateral FNACs of the testes in the workup of the azoospermia.

REVIEW OF LITERATURE

Defining Infertility

It is important to have knowledge of the normal human reproduction in order to define infertility. Studies of the pattern of conception in normal couples have shown that 60% to 75% of the couples will have a successful conception within 6 months of unprotected intercourse .The level will be 90% by 1 year [7]. The classic definition of infertility is based on this fact. Infertility is defined as the inability to conceive after 12 months of regular, unprotected intercourse. This definition was proposed by the Practice Committee of the American Society for Reproductive Medicine (ASRM) [8]. Based on this definition of infertility, it would therefore seem prudent to defer medical assessment of infertile couples until after 12 months of unprotected intercourse.

However, at the time of initial presentation, the performance of a basic evaluation of both partners is advised. This evaluation has to be cost effective. Currently the American Urological Association and the American Society for Reproductive Medicine recommend evaluation of infertility before 1 year if

(1) there is presence of history of bilateral cryptorchidism,

(2) female risk factors for infertility like advanced female age (older than 35 years) is present, or

(3) the male partner's fertility potential is in doubt.

Causes Of Male Infertility

The causes can be categorised as pre testicular, testicular and post testicular.

1. **Pre-testicular Causes**:

- Disorders of the hypothalamic or pituitary endocrine diseases (Thyroid or Adrenal disorders or Diabetes Mellitus)
- Metabolic disorders (Renal and Liver disease)
- Chronic Infection and Drugs

2. **Testicular Causes:**

- Idiopathic Hypospermatogenesis or aspermatogenesis
- Developmental and genetic disorders (agonadism, cryptorchidism, SCOS and Klinefelter's Syndrome).
- Circulatory: varicocele or torsion.
- Inflammatory lesions – infections or immune causes
- Iatrogenic: chemical, radiation or surgical

3. **Post-testicular causes (Genital)**

- Congenital : anomalies of excretory ducts or accessory glands.
- Acquired : Inflammatory lesions of the excretory ducts and accessory glands. Iatrogenic or post-traumatic lesions of the excretory ducts, accessory glands or ejaculation nerve plexus.

History Taking in the Evaluation of Male Infertility

It is essential to obtain a detailed history to make a successful diagnosis of infertility and to treat the infertility. Factors that can have a long term effect on fertility should also be considered. However, since human spermatogenesis involves an estimated sixty four day cycle with an additional time of five to ten days for epididymal transit, events that occurred in the recent past should also be given importance to. In patients who give a history of drug use, fever or illness in the past few months before semen testing , repeat testing should be done after an interval of three months .This is done to rule out detrimental effects that are transient.

It is important to take a thorough reproductive history. Details regarding any prior conceptions, duration of infertility, and use of contraception should be obtained. The definition of primary infertility is the failure to conceive at any time in the past .Secondary infertility is defined as the presence of infertility after a prior conception [9].

Paediatric conditions like mumps orchitis, cryptorchidism and testicular torsion or trauma that can affect eventual fertility should be obtained. The onset and timing of puberty may provide a clue to underlying endocrinologic abnormalities [10].

History of scrotal, inguinal, or retroperitoneal surgeries must be asked for. These procedures can lead to obstruction of vas deferens or affect emission or ejaculation.

History of diabetes mellitus, injuries to spinal cord, multiple sclerosis and disorders of the thyroid gland can affect ejaculatory as well as erectile function. These should be elicited in the history. Malignancies can lead to impaired spermatogenesis as a result of malnutrition, endocrinologic disturbances , fever with associated hyper metabolism, and immunologic factors. A detailed drug history, history of smoking and lung diseases, alcoholism etc should also be asked for.

Family history of infertility is also important in the initial evaluation of infertility and should be elicited. Finally, a complete history of female factor fertility should also include in the assessment. This is because about two thirds of infertility are due to abnormalities in the female partner, either wholly or in combination with male factors.

Examination Of Male patient with Infertility

Physical examination should be very thorough with special attention paid to the examination of genitalia. Body habitus, pattern of body hair and presence of gynecomastia etc. should be assessed. These provide clues to the adequacy of virilisation and deficiency of androgen. The thyroid

gland is thoroughly palpated. This may reveal nodules which suggest hyper function or hypo function of the gland. These can affect fertility[11].Hepatic dysfunction should be suspected if enlargement of liver is present on abdominal examination.

Careful examination of the phallus is an important part of examination of genitalia. The presence of chordee, abnormal curvature of penis or hypospadias should be looked for. These interfere with deposition of semen. The scrotal contents have also got to be carefully examined. Palpation of testes to assess its consistency and rule out tumour is also essential. Testicular size can be assessed with the aid of either an orchidometer, calipers, or by the use of ultrasound me. Normal average volume of adult testis is 20 ml [12]. Careful palpation of epididymis for enlargement or induration is essential. The spermatic cord should be examined in the supine and standing position. This allows the detection of varicocele. The vas deferens has to be carefully palpated. This is an important component of the assessment of spermatic cord. Last but not the least, a digital rectal examination should be done. This evaluates the prostate for midline cysts such as cysts of mullerian duct. These can obstruct the ejaculatory ducts. Induration of prostate or tenderness may be seen in acute or chronic prostatitis.

Laboratory Evaluation Of Male Infertility

1) Semen Analysis :

Analysis of semen is one of the most important tests to predict the potential for fertility in a male. There should be a minimum of two to seven days of sexual abstinence before collection [13]. Two separate samples at least seven days apart should be examined.

Masturbation is the recommended procedure for collection of specimen. Inaccurate results are obtained in samples collected by coitus interruptus. Hence this method of sample collection should not be encouraged. The specimen is collected in a clean and sterile container. The semen sample should be examined within one hour of collection .This is because some of the parameters can be affected by a delay in analysis. Motility decreases significantly after two hours. It progressively diminishes thereafter due to increase in activity of free radicals [14].

The characteristics that are analysed in the semen are classified into two groups: macroscopic and microscopic. The five macroscopic factors analysed include pH, Coagulation / Liquefaction, Colour, Viscosity and Volume. Microscopic examination of semen includes assessment of sperm agglutination, counts and concentration, motility, morphology, viability and looking for non sperm cells like epithelial cells, immature germ cells and leukocytes.

Computer-assisted sperm analysis (CASA) is a semi-automated Technique[14]. It provides information on various sperm parameters like sperm density, motility, flagellar beat frequency, straight-line and curvilinear velocity and hyper activation. In comparison to manual analysis, it offers two distinct advantages: high precision and quantitative assessment of kinematics of the sperm .However, equipment required for the test is expensive and hence it has not become standard of care.

Characteristics of Normal Semen (WHO, 2010)[15]

Parameter	Lower reference limit
Volume of semen (ml)	1.5 (1.4-1.7)
Total number of sperms (10^6 per ejaculate)	39 (33-46)
Concentration of sperm (10^6 per ml)	15 (12-16)
Progressive motility (%)	32 (31-34)
Morphology of sperm (normal forms, %)	4 (3.0-4.0)
Vitality (live spermatozoa, %)	58 (55-63)
pH	$\geq$ 7.2
Leucocytes	<1 million/ml
Total Fructose	>13 mmol/ejaculate
Micro Agglutination Reaction (MAR) test	<10% sperms with adherent particles

Acid phosphate	25,000 - 60,000 IU/ml
Zinc	90 - 600mg/100ml

Each variable, when taken alone, is neither a powerful nor the sole determining factor of fertility. The clinical setting and other parameters should also be taken into consideration when evaluating the importance of a particular factor.

2) **Sperm Function Assessment**

(i) *Sperm-Mucus Interaction/Postcoital Test*

The mature sperm must traverse the cervix and the cervical mucus in order for it to reach the site of fertilisation. The penetration of spermatozoa through cervical mucus demonstrated by in vitro tests is comparable to what occurs in vivo.

Postcoital test (PCT) is one of the methods to assess the interaction of sperm with cervical mucus and its migration. Cervical environment as a cause of infertility can be analysed by using this test. The timing of the test is very important and has to be accurate. The test must be carried out just be ovulation. This is when the cervical mucus is thin and clear. As part of the Post coital test, the cervical mucus is examined 2 to 8 hours after normal intercourse [16]. Presence of more than ten to twenty progressively motile sperm per high power field is considered normal. As per the guidelines of The American Society of Reproductive Medicine, PCT is

recommended in the following situations - hyper viscous semen, low-volume semen with normal sperm count or unexplained infertility [17] .

Couples who demonstrate a defect in interaction of sperm with cervical mucus, can be advised to proceed with intrauterine insemination (IUI). However, it must be noted that an abnormal post coital test can also occur as a result of inappropriate timing of the test, semen or cervical mucus antisperm antibodies, anatomic abnormalities, inappropriately performed intercourse, and abnormal semen [18]. If the postcoital test is persistently abnormal in the presence of reasonably good semen parameters it indicates very poor cervical mucus quality.

(ii) *Acrosome Reaction* :

Acrosome is a membrane-bound organelle. The anterior two third of the head of the sperm is covered by acrosome. Acrosome reaction is an exocytotic reaction. It involves fusion of the outer acrosomal membrane and sperm plasma membrane. It is an important step in fertilisation. This test is done when any defects in the morphology of sperm head is suspected. It may also be recommended in cases of unexplained infertility in patients with poor pregnancy rates with in vitro fertilisation. In normal semen samples, the spontaneous acrosome reaction rate is less than five percent .The induced acrosome reaction rate is between 15% to 40% [19]. In patients with infertility, spontaneous acrosome reaction rates are high while, rates of induced acrosome reactions are low [19].

(iii) *Sperm Penetration Assays/Sperm Zona Binding Tests* :

The sperm penetration assay (SPA) is a test to determine the functional capacity of the mature sperm to fertilize an oocyte. This test is based on the principle that a normal mature sperm has the ability to bind and penetrate the membrane of the oocyte[20]. This penetration has to occur for the sperm to fuse with the oocyte. Zona-free hamster eggs are used during the test. The test determines the ability of the sperm to undergo successful capacitation, acrosome reaction, fusion with oocyte membrane, and to undergo decondensation of chromatin. The assay is carried out by incubating sperm droplets and zone free hamster oocytes, for 1 to 2 hours. The oocytes are then examined under the microscope. Sperm penetration is looked for. This is indicated by swollen sperm heads within the oocyte cytoplasm. The test is considered normal if, ten to thirty percent of ova have been penetrated [20].

(3) **Advanced Semen Testing**

(i) *Antisperm Antibody (ASA) Testing* :

The tight junctions between the Sertoli cells form the blood -testis barrier. This barrier serves to prevent the immune system from coming in contact with the post-meiotic germ cells. In certain conditions like torsion of testis, trauma to testes and vasectomy, this barrier is violated [21]. As a result of this disruption, an immune response to sperm occurs. This leads

to development of antisperm antibodies. These antisperm antibodies are of several types— sperm immobilizing, spermotoxic or sperm agglutinating.

There are two types of antisperm antibody tests .The direct tests are used to detect sperms that are bound by immunoglobulins. The Indirect test is used to detect the biological activity of antisperm antibodies that are circulating.

(ii) *Electron Microscopy* :

Even if ultra structural defects are present, the spermatozoa may test positive for viability. Electron microscope is used to study the ultra structural details of the sperm[22]. Defects in the mitochondria and microtubules can be detected by electron microscopy.

(iii) *Biochemical Tests* :

Acrosin, is a serine protease-like enzyme. It exhibits a lectin-like carbohydrate binding activity to the glycoproteins of oocyte zona pellucida. Low sperm density, motility, and poor morphology can occur if the levels of acrosin are low.

Zinc is essential for stability of the chromatin, decondensation and for head–tail detachment during fertilization. Zinc levels can be measured by colorimetric methods .The reference value is 13 mmol per ejaculate[23]. In asthenospermia and oligoasthenospermia, the level of zinc in seminal plasma is decreased. However zinc levels are increased in spermatozoa.

The bulk of seminal fluid volume is contributed by secretions from the seminal vesicles. This fluid serves as the transport medium for sperm. It also contains fructose which is essential for nutrition of the sperm. The level of fructose in seminal fluid correlates well with sperm motility. Fructose is absent or its levels are low in conditions that cause ductal obstruction and also congenital conditions like Congenital Bilateral Absent Vas Deferens.

Secretions from the epididymis contain L- carnitine. The levels of L-Carnitine in seminal plasma are up to ten times that present in the serum .L-Carnitine plays a role in maturation of sperm[24]. Low levels of L-Carnitine are found in men having oligoasthenospermia.

Alpha glucosidase, a specific marker for epididymal function, is essential for maturation of sperm during the epididymal transit. A cutoff value of 12 mIU/ml can be used to differenitate ductal obstruction from primary testicular failure [21].

(iv) *Reactive Oxygen Species* :

Reactive oxygen species (ROS) and free radicals can be produced in excessive amounts. This may lead to sperm damage and abnormalities in seminal parameters. Several tests are available to ascertain the levels of excessive reactive oxygen species as well as its source of generation in semen. But these tests are not currently done as part of the routine evaluation of the infertile male. If, however, high level of reactive oxygen

species are noted, it serves an independent marker of infertility in the male [25]. This is especially so if the samples are leukocytospermic after adjustment for semen characteristics.

(v) *Sperm DNA Damage* :

The mature spermatozoa contain chromatin that is tightly packed. This is because of the disulfide cross linkages that exists between proteins .As a result of this tight packaging there is compaction of the nuclear head. This in turn serves to protect the DNA fragments from stress and breakage.

DNA damage can occur as a result of several factors. Some of the factors associated with DNA damage include protamine deficiency and mutations of the DNA that alter its packaging or compaction during spermiogenesis. There is good correlation between poor seminal parameters and DNA damage [26]. This is especially so if the concentration of sperm is low, there is low sperm motility, leukocytospermia, and oxidative stress. Many tests to assess DNA damage in sperms are available. DNA damage in sperms can be measured by direct (fragmentation, oxidation) as well as indirect tests (sperm chromatin compaction).

Direct tests to assess DNA damage include single cell gel electrophoresis assay or “comet” assay, liquid chromatography test which measures DNA oxidation levels and terminal deoxynucleotidyl transferase mediated dUTP-nick end-labelling or “TUNEL” assay.

Indirect tests to assess sperm DNA damage are sperm chromatin integrity assays and tests to evaluate levels of nuclear protein.

(4) **Endocrine Evaluation** :

Endocrine causes are uncommonly found in male sub fertility Endocrinopathies are observed in up to three percent of men with infertility[27]. Some authors recommend routine evaluation of the hypothalamic-pituitary-gonadal axis in all male patients with infertility. The consensus, however is to do an endocrine evaluation in infertile males with either of the following indications :

(1) Very low concentration of sperm ,i.e., less than 10 million/ml;

(2) Sexual function is impaired

(3) other findings that suggest the presence of endocrinological cause for male infertility such as a marked reduction in size of testes or presence of gynecomastia.

Measurement of Follicle-stimulating hormone (FSH) and morning serum testosterone is the first step in hormonal evaluation[18]. Both FSH, LH and testosterone are secreted in a pulsatile manner. Since there is a normal physiologic decline in the levels of testosterone throughout the day, early morning samples are preferred.

Normally, follicle stimulating hormone secretion is under the negative feedback control of inhibin B, which is produced by the Sertoli cells.

An elevated level of serum FSH is seen in disturbances of spermatogenesis such as primary testicular failure. Normal levels of FSH, LH and testosterone are noted in obstructive azoospermia. Hypogonadism as result of pituitary or hypothalamic causes are associated with low testosterone levels. Low levels of testosterone are also noted in primary testicular failure.

If the initial endocrinologic evaluation is abnormal, further tests are carried out in the form of a repeat testosterone assay and assays of serum luteinizing hormone (LH) and serum prolactin levels. Free as well as total testosterone levels are also done.

Low levels of follicle stimulating hormone and luteinising hormone indicate the possibility of hypogonadotropic hypogonadism as in Kallman syndrome .A complete pituitary hormonal assessment including thyroid stimulating hormone, growth hormone and adrenocorticotropic hormone assays are done in such cases.

Mild elevations (<fifty ng/ ml) of serum prolactin can occur as a result of drugs, renal insufficiency, stress or it can be idiopathic. However, if the level of prolactin is elevated even in repeat testing, a pituitary tumour such as a prolactinoma should be suspected and examined for [27].

Elevated levels of oestrogen may lead to gynecomastia, erectile dysfunction and diminishes sexual desire. The serum testosterone levels may also be low.

Thyroid function tests are not done as part of the routine in a male with infertility. It is recommended only in patients with clinical features of thyroid dysfunction.

(5) **Genetic Testing** :

Genetic tests in an infertile male are done to establish the cause of infertility, and to identify other potential medical issues in the patient. They are also done to predict the efficacy of various therapeutic interventions such as varicocele repair and to provide information to couples regarding transmission risks to offspring during counselling. Karyotyping and y-linked microdeletion assessment form part of the genetic tests done in a male with infertility.

Imaging studies in the Evaluation of Male Infertility

Radiographic imaging studies are done in the infertile male to demonstrate genital tract obstruction in the vas deferens or ejaculatory duct if any. They are also done to rule out associated pathologies such as testicular masses or renal abnormalities.

(i) *Transrectal Ultrasonography (TRUS)* :

This test provides a very good assessment of the prostate, seminal vesicles, ampulla of the vas deferens, as well as the ejaculatory ducts [28]. A five - to seven -MHz endo cavitary probe is employed. Scanning is done in both the transverse plane as well as the longitudinal plane.

Transrectal ultrasound is mainly used to assess patients with suspected ejaculatory duct obstruction. Patients with ejaculatory duct obstruction usually have low semen volume (less than one ml) associated with azoospermia, low seminal pH and absence of fructose in semen. Width of seminal vesicle in excess of twelve to fifteen mm or diameter of ejaculatory duct that is greater than 2.3 millimetre is considered suggestive of obstruction [29].

Seminovesiculography is done under transrectal ultrasound guidance. Radioopaque contrast (50% renograffin) is injected transrectally into the seminal vesicles. Post injection radiographs are taken. These pictures provide a very good anatomic detail of the seminal vesicles and ejaculatory ducts.

(ii) *Scrotal Ultrasonography* :

Ultrasound of the scrotum is primarily done in an infertile male to confirm the presence of clinically detected varicoceles. High-quality imaging of other scrotal contents such as testes and epididymis are also obtained. Duplex sonography is usually done when the clinical examination for varicocele is equivocal or is difficult due to body habitus. Presence of varicocele is indicated by presence of reversal of venous blood flow with the Valsalva manoeuvre. A spermatic vein diameter of greater than three millimetres also suggests the presence of varicocele [30].

Scrotal ultrasound also provides excellent anatomic details of the epididymis and testis. A number of conditions that may affect fertility in a male like epididymitis, testicular germ cell tumors and epididymal cysts can be diagnosed .

(iii) *Abdominal Ultrasonography* :

Ultrasound of the abdomen is primarily done in a n infertile male to rule out associated renal anomalies in patients with agenesis of vas deferens. Upto twenty percent of men with agenesis of vas deferens can have anomalies in the ipsilateral kidney. This is most likely to be renal agenesis [13].

(iv) *Vasography* :

The gold standard test to verify the patency of the ductal system in an infertile male is Vasography[31]. It is done to determine the site of obstruction in the patients with azoospermia on semen analysis but have normal spermatogenesis demonstrated in testicular biopsy.

The ideal time to perform a Vasography is just before planned surgical reconstruction. This is because, this test has the potential to cause vasal scarring at the site of contrast injection. Contrast is injected into the vas by means of either a puncture or vasotomy. The puncture technique is preferred. This technique avoids a full-thickness vasotomy, which then requires subsequent microsurgical closure.

Azoospermia

Azoospermia, by definition, is the absence of sperm in the ejaculate. It is identified in ten to fifteen percent of males with infertility[32]. Even if a small quantity of sperm is identified in the centrifuged specimen the possibility of complete ductal obstruction such as Congenital Bilateral Absence of Vas Deferens (CBAVD) can be ruled out.

Pre testicular causes (secondary testicular failure) for azoospermia are usually hormonal in nature. It can be due to either congenital hypogonadotropic hypogonadism (Kallman syndrome) or acquired hypogonadotropic hypogonadism. Testicular causes for azoospermia (primary testicular failure) are due to intrinsic defects in spermatogenesis. Post testicular pathologies like ejaculatory dysfunction or obstruction of the genital tract can also lead to azoospermia.

High levels of serum follicle stimulating hormone (greater than two times normal) are indicative of primary testicular failure. Testicular biopsy to rule out obstructive causes for infertility is not required in such a condition.

Azoospermia associated with primary testicular failure is referred to as *nonobstructive azoospermia* (NOA). It is best managed with harvesting of testicular sperm for Intracytoplasmic sperm injection.

Diagnostic testicular biopsy is required in patients with azoospermia in whom the testicular size is normal, vas deferens is palpable, and the

serum levels of follicle stimulating hormone are normal. Testicular biopsy is done in such cases to differentiate azoospermia from obstruction of genital tract from other disorders of spermatogenesis such as maturation arrest. A normal testicular biopsy in an azoospermic male is pathognomonic for obstruction of the genital tract. These patients may then have to undergo exploration of the scrotum and vasography to identify the exact site of obstruction.

Testicular Biopsy in the Evaluation of Infertile Male

The role of testicular biopsy in the management of male infertility is twofold :

(i) It is used as a diagnostic tool to differentiate obstruction from non-obstructive pathology.

(ii) For therapeutic purposes to harvest sperm with the intention for use in ICSI.

Diagnostic testicular biopsy is indicated to evaluate azoospermia[19] in patients who present to infertility clinics with a clinical picture that is suggestive of genital tract obstruction. The size and consistency of the testes should be normal as should the serum follicle stimulating hormone levels. However, a diagnostic testicular biopsy can still be done in a patient with clinical evidence of testicular failure as indicated by small-volume of the testes and high levels of serum follicle stimulating hormone. Testicular biopsy in such situations assesses the ability to perform harvesting of

sperm for Intracytoplasmic injection in the future. In this setting, therefore testicular biopsy should be combined with sperm extraction and cryopreservation. This also avoids the need for a repeat biopsy in the future.

Men with known causes of genital tract obstruction such as those with a history of vasectomy or agenesis of vas deferens do not require a testicular biopsy [33]. Biopsy of one of the testis is usually sufficient to assess the for obstruction in an azoospermic male. Testicular biopsy specimen obtained is placed in special solutions such as Bouin's, Zenker's, or buffered glutaraldehyde. The usually used formalin preservative will introduce distortion artefacts into the specimen. This makes analysis of the specimen less accurate.

Testicular biopsies have to be interpreted by an experienced pathologist. This is because, the analysis is descriptive rather than quantitative in nature.

Testicular FNAC in the Evaluation of Infertile Male

Testicular FNAC is only beginning to gain acceptance as a diagnostic modality in patients with azoospermia. It is greatest value in the evaluation of Non Obstructive Azoospermia. In such situations, it can conserve tissue in an already failing organ. FNAC of the testes was first described by Max Hubner in 1965 [5]. However it did not gain popularity

back then due to the lack of awareness and technical expertise to interpret the cytological data.

If at least 200 cells could be counted on minimum in one well spread slide, the specimen is considered adequate[34]. Approximately 97% testicular FNAC's yield adequate specimen for evaluation of spermatogenesis.

Advantages of FNAC include the fact that it is quick, simple and can be done as an outpatient procedure. Complications are rare and there is good concordance between histology and cytology. In addition, the material obtained can be used to quantify spermatogenesis by DNA flow cytometry.

However, testicular FNAC has its own disadvantages. It can't provide information on the architecture of the testes nor on the thickness of basement membrane and interstitial tissue. A fairly experienced cytopathologist is required to interpret the findings.

Interpretation of Testicular Biopsy / FNAC

Diagnosis involves two steps:

(i) Identification of the cell types present

(ii) The proportions of the cell population represented by each.

Two cell populations can be recognised in cytology samples - the first are Sertoli cells and the second are cells in various stages of spermatogenesis. The spermatogenetic cells are divided into

(i) Spermatogonia, (ii) primary spermatocytes, (iii) secondary spermatocytes, (iv) spermatids and (v) spermatozoa.

Cytological features of these cells are described below:

A) Sertoli cells: They have round or oval nucleus with a rather smooth chromatin pattern. Large pale or blue nucleoli are usually present. There is abundant cytoplasm in a Sertoli cell. The cytoplasm is pale slate blue and is usually foamy with ill-defined borders. Although occurring singly, these cells usually form sheets or a loose matrix. Sertoli cells are invariably present even in the total absence of spermatogenesis.

B) Spermatogenetic cells (in order of maturation):

(i) Spermatogonia: They contain 16-20 μ m round or oval, slightly eccentrically placed nucleus with smooth finely condensed nuclear chromatin which is either pale staining (light) or dark staining. Nucleoli are not usually seen. The cytoplasm is homogenous and has well defined border.

(ii) Primary Spermatocytes: It is the largest germ cell. It contains a round nucleus, 14-20 μ m, the size depending upon the state of maturation, with a heavy coarse chromatin pattern. The nuclear chromatin shows a 'chunky' appearance with a clear dark/light effect. The cytoplasm stains deeply hyper-basophilic and is moderate in amount. Nucleoli are not seen.

(iii) Secondary Spermatocytes: It contains a round nucleus, variable in size depending on maturation; from 8-16 μm. Binucleate forms are common. The chromatin pattern is coarse but to a lesser extent than that seen in the primary spermatocytes. The cytoplasm is moderate in amount, basophilic but not hyperbasopholic as seen in the primary spermatocyte. Nucleoli are not seen. These cells are rarely recognised because of their shorter life span and immediate transformation to spermatids.

(iv) Spermatids: A small cell, although the size is variable. The nucleus is less than 8 μm depending, as in the other spermatogenic cells, upon maturation stage. In the ′close to mature′ stage, the nucleus of course resembles a sperm head. The nuclear chromatin is darkly staining and smooth. The cytoplasm is grey blue and often shows a ragged, uneven border. Sperm tails are commonly seen either in or protruding from the cytoplasm.

(v) Mature Spermatozoa: The nuclei of mature spermatozoa are oval with very dense chromatin. The tail is found on side opposite to the acrosome. This end point is proof that, spermatogenesis, the transformation of spermatid to spermatozoa, is functional.

(C) Leydig (interstitial) cells: Leydig cells are relatively uncommon in testicular cytology when compared with the other cellular components. They are however usually present in small numbers if careful scrutiny is

applied. The nucleus is 10-12 μm, round, darkly staining with a relatively smooth chromatin. The cytoplasm is abundant and stains basophilic. The cell borders are usually clearly defined in contrast to the poorly defined Sertoli cell borders.

The cytoplasm is also cleaner and smoother when compared with Sertoli cells. Scattered green/blue granules are seen lying within the cytoplasm.

(D) Mesothelial cells:

Mesothelial cells are an expected finding, picked up on the way in from the scrotal lining and occur in large monolayer sheets with moderately high N/C ratio. They have moderate amount of bluish cytoplasm & well demarcated cell borders, large nuclei & prominent nucleoli. Cytoplasmic vacuoles may be seen.

Based on various proportions of the different cell types, the smear is categorized into five groups [1]:

(i) Normal spermatogenesis – This pattern is reported when the smears show spermatogonia, spermatocytes, spermatids, many spermatozoa and a proportional numbers of Sertoli cells forming roughly one third of the total spermatogenetic cells. The process of spermatogenesis occurs in an orderly fashion. Spermatogonia are present along the basement membrane and then there are the spermatocytes in between and finally mature spermatozoa are found adjacent to the tubular lumen. In the setting of

azoospermia, a normal testicular biopsy is considered pathognomonic of ductal obstruction.

(ii) Hypo spermatogenesis – This pattern is described when all types up to spermatozoa are present and the proportion of Sertoli cells to spermatogenic cells is increased. Though there is a decrease in the number of all germ cells, all the various stages of spermatogenesis are present in the histologic section.

(iii) Maturation Arrest - In this condition there is a block in the maturation of sperm at a specific stage. This arrest can occur anywhere along the path of spermatogenesis. Maturation arrest most commonly occurs at the stage of primary spermatocyte or late spermatid stage. Complete maturation arrest results in absence of sperm in semen. In patients whom the maturation arrest is partial, severe oligospermia is noted.

(iv) Germinal Aplasia - This is also referred to as Sertoli cell-only syndrome. The seminiferous tubules are small. They are completely devoid of germ cells. However, the testicular interstitium, Sertoli cells and basement membranes, are normal. The diagnosis is suspected in a patient with azoospermia in whom the Testes are small and levels of follicle stimulating hormone are high. This indicates primary testicular failure.

(v) Testicular / Tubular Atrophy (End Stage Testis)– It is characterised by basement membrane thickening, sclerosis of tubular and peritubular region, and absence of both germ cells and Sertoli cells. Absence of sperm in semen with small, soft testes is noted in such patients.

Testicular Fine Needle Aspiration versus Testicular Biopsy

FNAC is capable of recovering sufficient material for cytological assessment of infertility as compared with biopsy. In fact, there have been many reports claiming that FNAC is superior to the "gold standard" testicular biopsy [9]. FNAC at times provides information not evident on histology. This is mainly because of the heterogeneity of the testes, the organ may contain more mature germ cell lineage in small foci far from the site of biopsy. A wider area of testes can be sampled by Fine needle aspiration cytology. This in turn results in a more accurate representation of the true histological geography and variation that exists within the testis. Despite a histological finding of no germ cells within the seminiferous tubules, clinical experience in men undergoing testicular sperm extraction and ICSI has demonstrated that even testes with such histological findings can harbour fertile sperm. This fact underlines 2 practical points - the importance of careful examination of a testicular biopsy to find mixed histological patterns of infertility and also the need

for wider geographic sampling[35]. The latter can be achieved more practically by FNAC than with multiple biopsies.

Many studies have revealed the concordance rate between FNAC and histological diagnosis to be more than 85% with specificity as high as 95%[6].

Some of the advantages of FNAC are that reporting can be quicker as compared to biopsy. Complications related to the procedure are rare. It is simple, quick and inexpensive because surgical instruments are not required. Local scarring does not occur. It is very well tolerated by the patient. The material obtained shows excellent preservation and various cell types can be identified. FNAC guided Testicular Epididymal Sperm Extraction (TESE) is a useful alternative to blind biopsy [36].

There are also some limitations to FNAC .It is unable to provide information on architecture of the testes. It does not give information about thickness of the tubular basement membrane, tubular diameter or status of the interstitial tissue. Testicular disorders leading to azoospermia such as atrophy, fibrosis and Leydig cell hyperplasia are better diagnosed on the basis of histology. Some patients also complain of prolonged pain but this is relieved by scrotal support and analgesics. Fairly experienced pathologists are needed to interpret the smears. Neurogenic shock has been reported [37] in patients who failed to rest after the procedure. Hematoma formation can be expected when a thick gauge needle (20G) is used.

MATERIAL AND METHODS

Study Design: Prospective study

Duration : October 2011 to Feb 2013

Setting: Govt. Stanley Medical College and Hospital, Chennai.

Inclusion Criteria:

1) Infertile men with two consecutive semen samples showing azoospermia; tests done at least two weeks apart (All semen analysis done only after a period of abstinence of at least 4 days).

Exclusion Criteria:

1) Infertile men with only a single sample showing azoospermia.
2) Infertile men with normal sperm counts or oligospermia.
3) Infertile men with azoospermia in whom testicular biopsy /FNAC is contraindicated due to causes like bleeding diathesis, infection etc.

A total of 42 patients who fitted into the inclusion and exclusion criteria were enrolled in the study after getting a proper informed consent.

Methodology:

All infertile men having two successive semen samples showing azoospermia attending the Urology OPD who volunteer for study are included after informed consent. A clinical examination is then conducted and relevant personal and clinical data are noted. The patients are then

investigated with hormonal assays and scrotal ultrasound as per Performa, in addition to the routine investigations. Patients were then subjected to FNAC of both the testes for cytological evaluation and open testicular biopsy was done for histopathological correlation. The choice of testis for biopsy depended on the clinical examination. The larger testis was chosen for biopsy and in case of similarity in size, the choice was random. The patients were discharged on the same day of the procedure with prescription for appropriate antibiotics and analgesics. The biopsy and FNAC reports were reviewed and correlated.

Considering histopathological examination as the gold standard, in case of discordance between the FNAC reports of both testes, the one that correlated with the histopathological report was considered for statistical analysis.

FNAC technique:

The procedure was done in the Urology OT (Operation theatre) at Govt. Stanley Hospital and Medical College. The patient is placed in the supine position, and the scrotum was palpated to confirm the clinical findings. Cord block was then given by injecting 5 ml of 1% xylocaine. Five minutes after injection of local anaesthetic, the testis was held with the left hand and was positioned with epididymis present posteriorly. The testis was gently held between the thumb and the index finger. Using the right hand, the testis was punctured with a 22 gauge needle attached to a 10 ml

disposable syringe. Needle was passed in an axis perpendicular to the longitudinal axis of testis. With to and fro movements of the needle, aspiration was done twice or thrice under negative pressure. Negative suction was released .Tissue fragments obtained were then expelled onto a clear glass slide and gently smeared. Open testicular biopsy was performed immediately following the procedure of FNAC of the testis. Smears were air-dried and fixed with 95% alcohol. They were then sent for cytological examination. The procedure was then repeated on the opposite side.

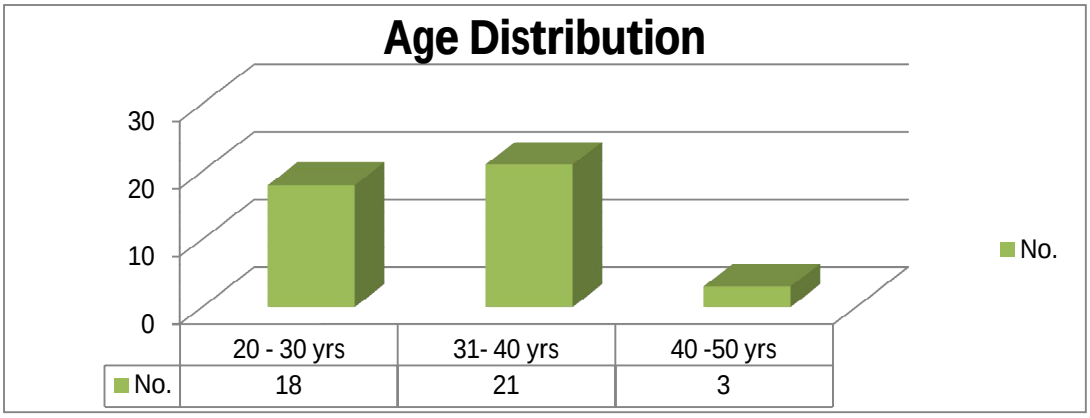
Testicular biopsy technique:

A one centimetre incision was made on the centre of the anterior portion of the larger testis, through the portal of entry of the FNA needle. The incision is deepened through the skin and dartos up to the tunica albuginea. Gentle pressure was then applied on the testis. This resulted in extrusion of a small amount (2 to 3 mm) of testicular parenchyma from the incision. This was then excised with a sharp pair of curved Iris scissors. The tissue obtained was then transferred to a bottle containing Bouin‘s fluid. After securing homeostasis, the tunica albuginea, tunica vaginalis, dartos and skin were closed in layers with 3/0 vicryl sutures. A dry scrotal dressing was then applied. The specimen was labelled and sent for histopathological examination.

RESULTS

1) Age Distribution

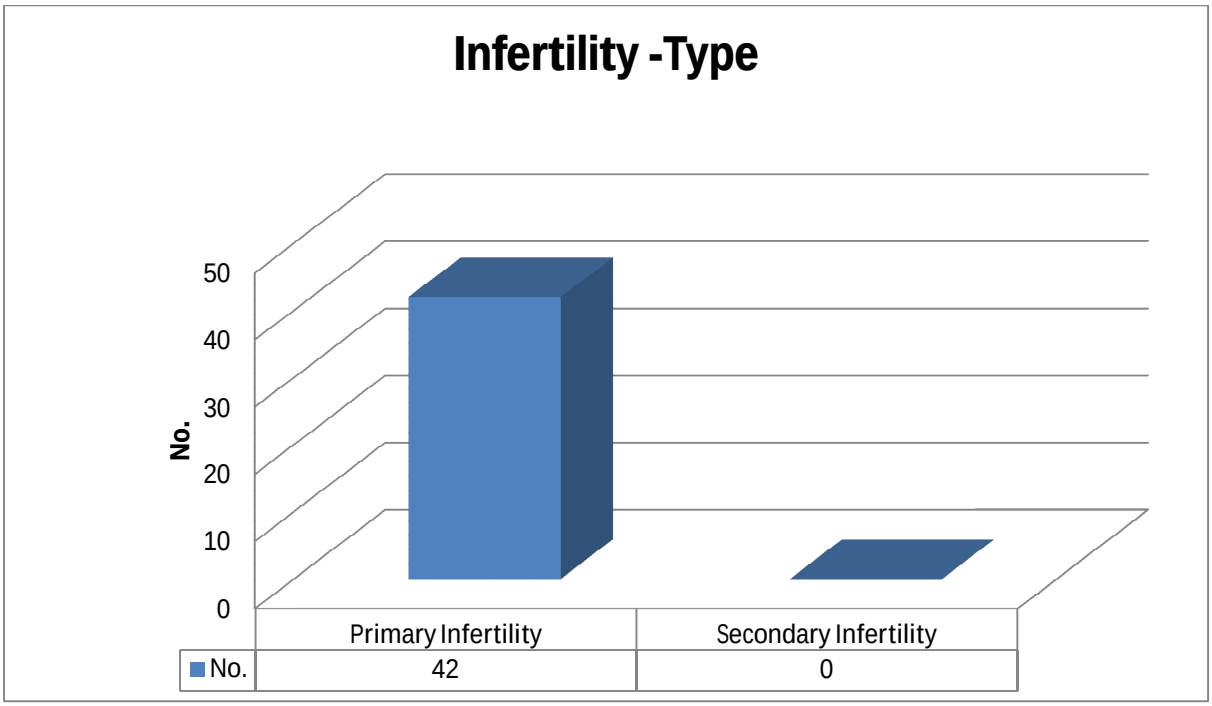
Age Range	No.	Percentage of Total
20 - 30 yrs	18	42.80%
31- 40 yrs	21	50%
40 -50 yrs	3	7.20%



There were 42 patients in the study with age range between 25 to 43 years. The mean age of the study population was 31.83 years. The majority of the study population was in the 31 -40 yrs age group (50%). The most in a particular age was 7 in the 31 year age category.

2) Infertility – Type

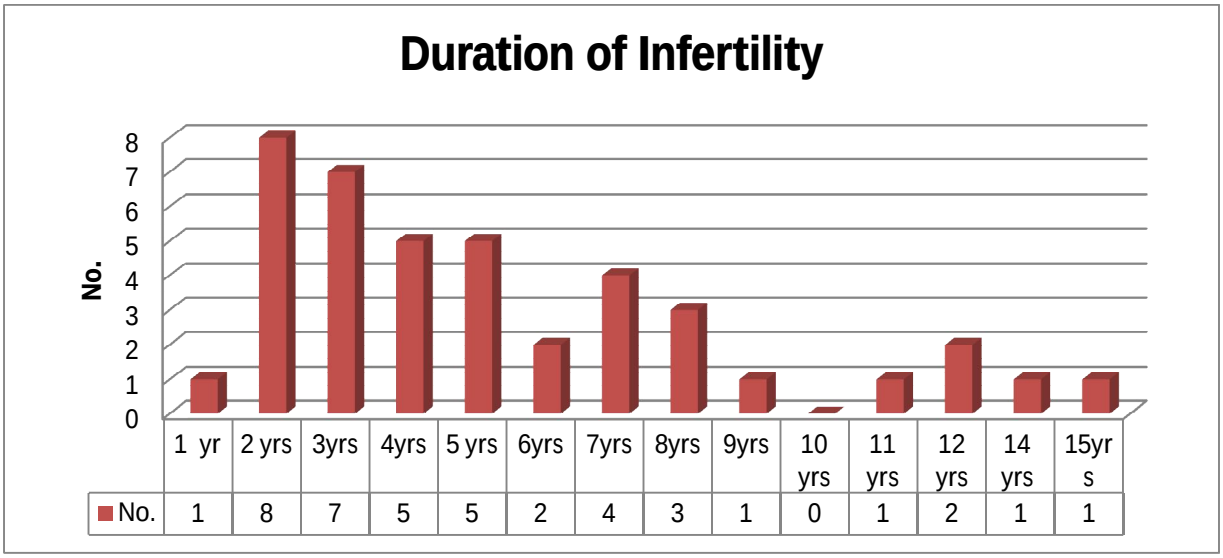
Infertility	**No.**	**Percentage**
Primary Infertility	42	100%
Secondary Infertility	0	0%



The infertility was primary, in all (100%) the patients studied. There were no patients with secondary infertility in the study.

3) Duration of Infertility

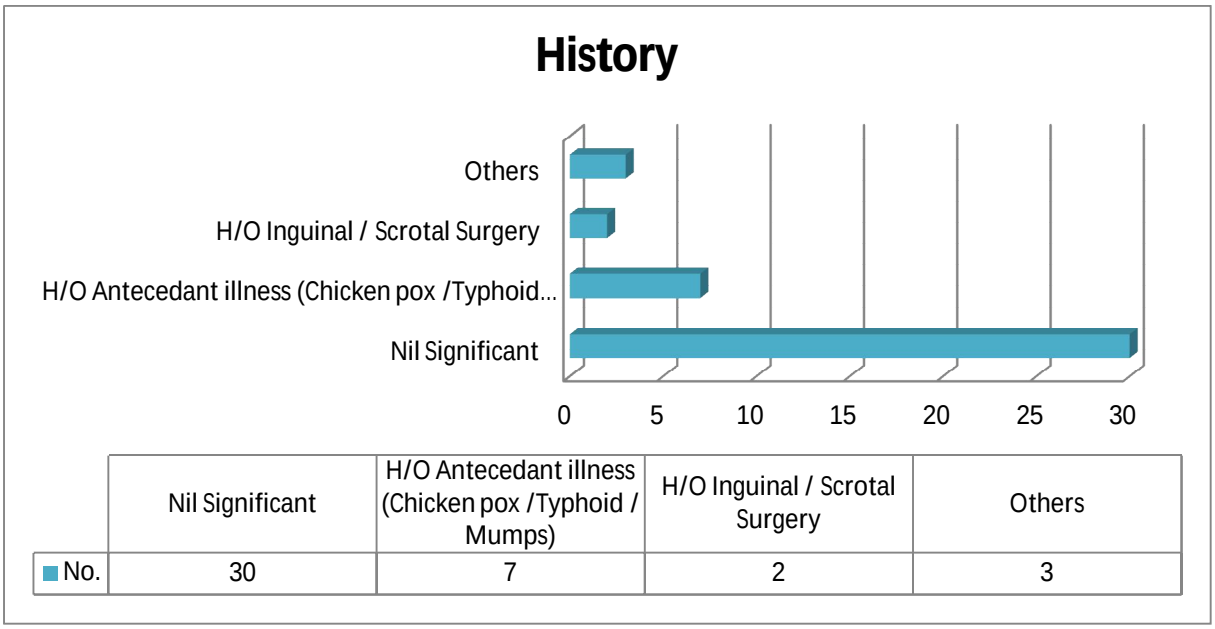
Duration of Infertility	No.	Percentage
1 yr	1	2.40%
2 yrs	8	19.10%
3yrs	7	16.70%
4yrs	5	11.90%
5 yrs	5	11.90%
6yrs	2	4.80%
7yrs	4	9.60%
8yrs	3	7.10%
9yrs	1	2.40%
10 yrs	0	0%
11 yrs	1	2.40%
12 yrs	2	4.80%
14 yrs	1	2.40%
15yrs	1	2.40%
20yrs	1	2.40%



The duration of infertility in our study group ranged from 1 – 20 years. The mean duration was 5. 72 years. There were a maximum of 8 patients (19.1%) who presented with a history of 2 year duration of infertility followed by 16.7 % (7 patients) who presented with infertility of 3 year duration.

4) History

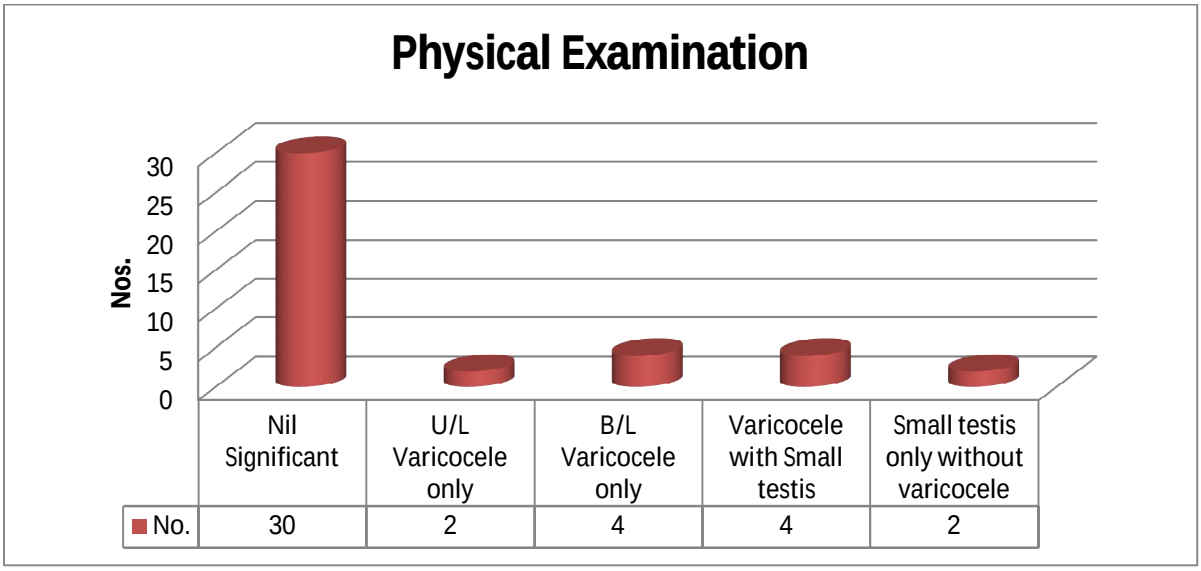
History	No.	Percentage
Nil Significant	30	71.40%
H/O Antecedant illness (Chicken pox /Typhoid / Mumps)	7	16.70%
H/O Inguinal / Scrotal Surgery	2	4.80%
Others	3	7.10%



Of the 42 patients in our study, a majority i.e. 71.40 % (30 patients) did not have a significant history. In the remaining 12 patients, 7 (16.70 % of the study population) had history of febrile illness – mumps ,chicken pox or typhoid. There were 2 patients with a history of inguinal / scrotal surgery.

5) Physical Examination

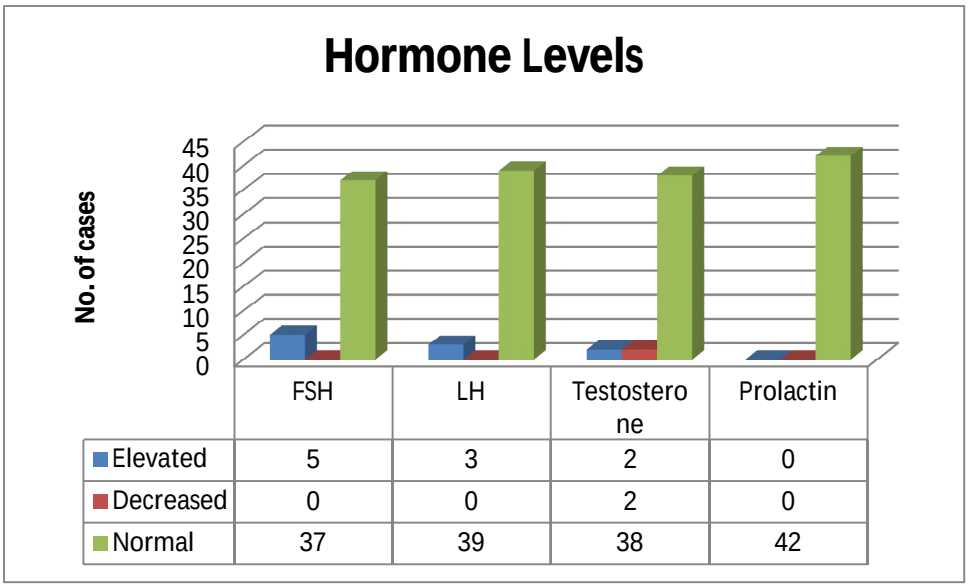
Findings	No.	Percentage
Nil Significant	30	71.40%
U/L Varicocele only	2	4.80%
B/L Varicocele only	4	9.60%
Varicocele with Small testis	4	9.60%
Small testis only without varicocele	2	4.80%



Similar to the history aspect, an identical number of patients (30 ;71.40%) did not have any significant finding on physical examination. Of the remainder, varicocele was noted in 10 patients (23.8%) – 2 were unilateral, 4 were bilateral and 4 patients had a small testis in addition to the varicocele.

6) Hormone Levels

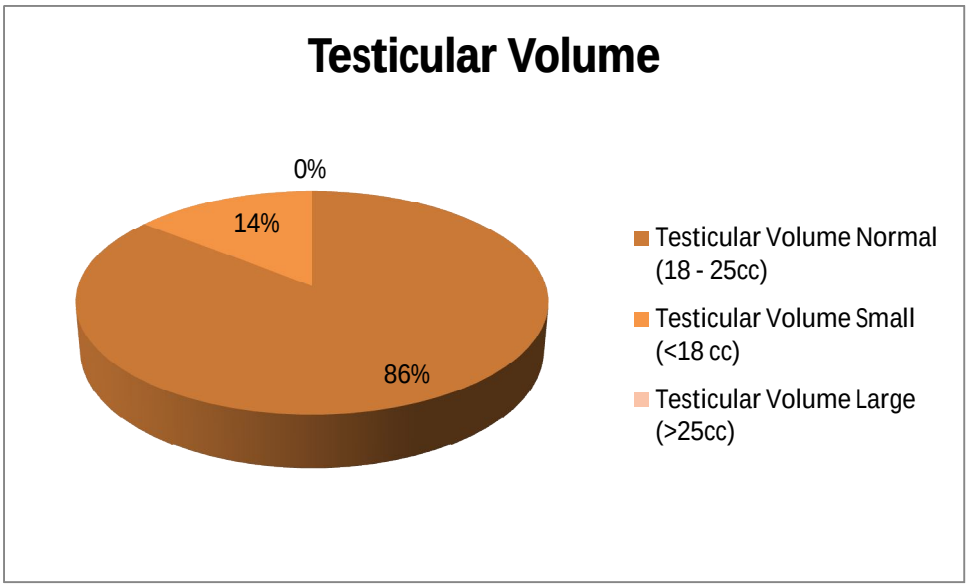
Hormone	Elevated	Decreased	Normal
FSH	5 (11.9%)	0	37 (88.1.%)
LH	3 (7.1%)	0	39 (92.9%)
Testosterone	2 (4.8%)	2 (4.8%)	38 (90.4%)
Prolactin	0	0	42 (100%)



The majority of the patients in the study group had normal hormonal levels .Of the 42 patients, 5 (11.9%) had elevated FSH levels, 3 (7.1%) had elevated LH levels, 2 (4.8%) had elevated testosterone and none had elevated Prolactin. In addition two patients (4.8%) had decreased Testosterone. None of the patients had decreased FSH, LH or Prolactin levels.

7) Testicular Volume

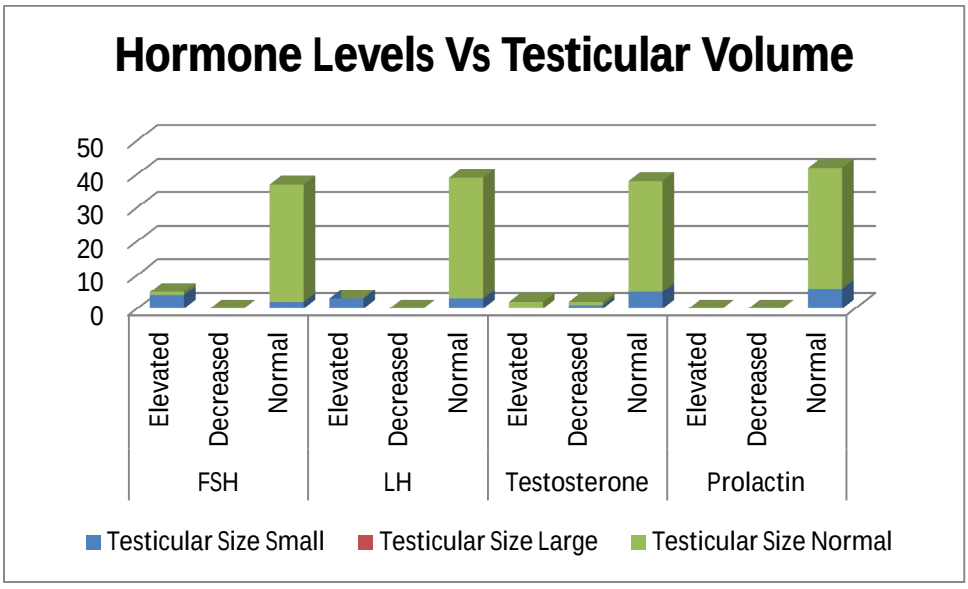
Testicular Volume		
Normal (18 - 25cc)	**Small (<18 cc)**	**Large (>25cc)**
36 (86%)	6 (14 %)	0



86% of the study population had normal testicular volume while 14 % had a small testes (< 18 cc).

8) Hormone Levels Vs Testicular Volume

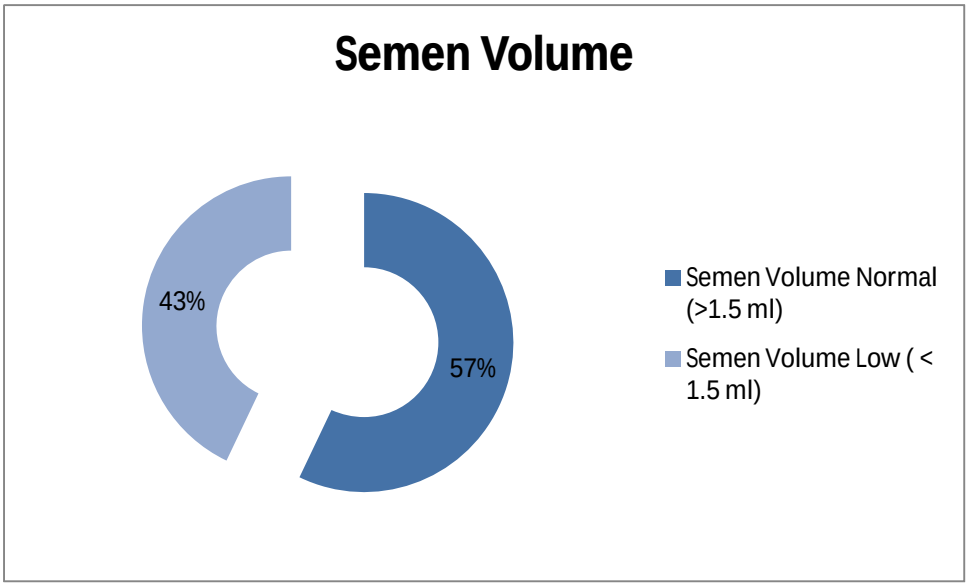
Hormone	Level	Testicular Size		
		Small	Large	Normal
FSH	Elevated	4 (9.5%)	0	1 (2.4%)
	Decreased	0	0	0
	Normal	2 (4.8%)	0	35 (83.3%)
LH	Elevated	3 (7.1%)	0	0
	Decreased	0	0	0
	Normal	3 (7.1%)	0	36 (85.7%)
Testosterone	Elevated	0	0	2 (4.8%)
	Decreased	1 (2.4%)	0	1 (2.4%)
	Normal	5 (11.9%)	0	33 (78.6%)
Prolactin	Elevated	0	0	0
	Decreased	0	0	0
	Normal	6 (14.3%)	0	36 (85.7%)



Among the 5 patients with elevated FSH, 4 (80%) had small testes. All 3 patients with elevated LH had small testes. Of the two patients with decreased testosterone, 1 (50%) had small testes. Hence in our study, majority of the patients with elevated FSH and LH and with decreased testosterone had small testes.

9) Semen Volume

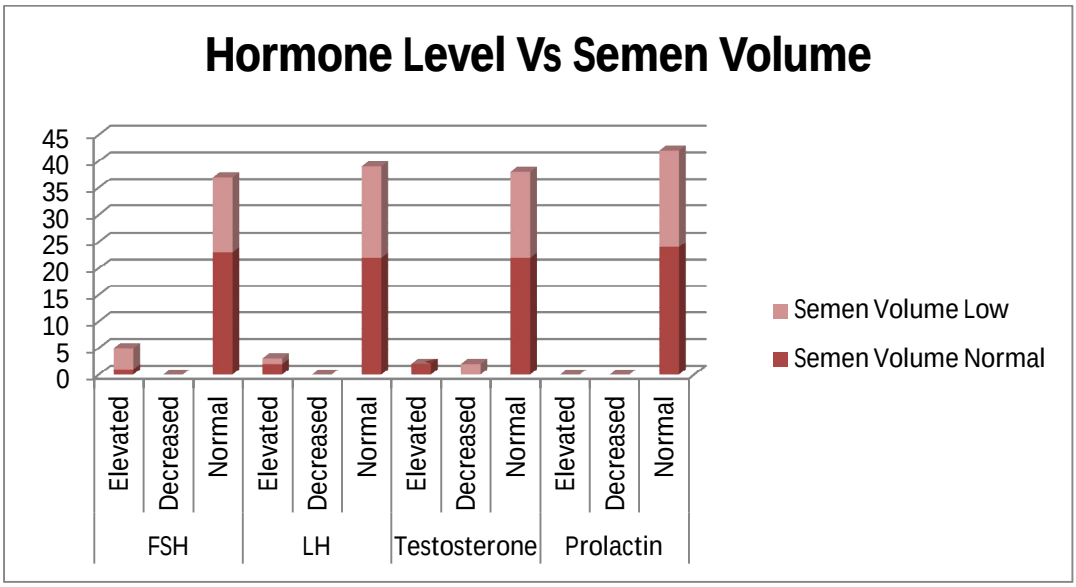
Semen Volume	
Normal (>1.5 ml)	**Low (< 1.5 ml)**
24 (57%)	18 (43 %)



Of the study population, 43% (18 patients) had a semen volume of less than 1.5 ml.

10) Hormone Levels Vs Semen Volume

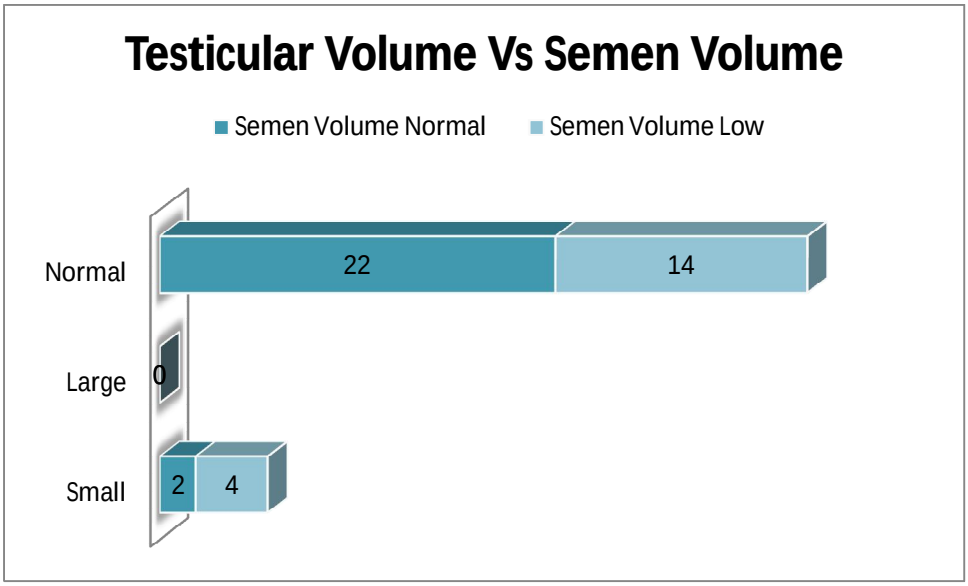
Hormone	Level	Semen Volume	
		Normal	Low
FSH	Elevated	1 (2.4%)	4 (9.6%)
	Decreased	0	0
	Normal	23 (54.8%)	14 (33.3%)
LH	Elevated	2 (4.8%)	1 (2.4%)
	Decreased	0	0
	Normal	22 (52.4%)	17 (40.5%)
Testosterone	Elevated	2 (4.8%)	0
	Decreased	0	2 (4.8%)
	Normal	22 (52.4%)	16 (38.1%)
Prolactin	Elevated	0	0
	Decreased	0	0
	Normal	24 (57 %)	18 (43%)



Of the 5 patients with elevated FSH, 4 (80%) had decreased semen volume. 1 of the 3 patients (33%) with elevated LH had decreased semen volume, while both the patients (100%) with decreased testosterone had a semen volume of less than 1.5ml. Hence in our study, majority of the patients with elevated FSH, LH and decreased testosterone had low semen volumes.

11) Testicular Volume Vs Semen Volume

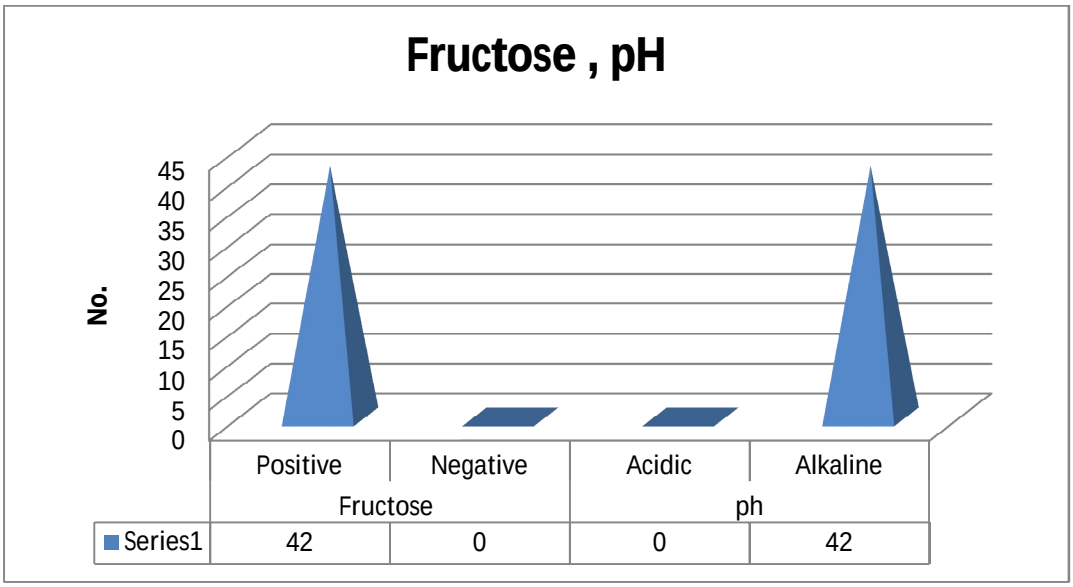
Testicular Volume	Semen Volume	
	Normal	Low
Small	2	4
Large	0	0
Normal	22	14



Of the 6 patients with small testes, 4 (66 %) had a semen volume of less than 1.5ml. 14 of the 36 patients (39%) with normal testes had a decreased semen volume. In the study, the incidence of low semen volume was higher in patients with low testicular volume when compared to those with normal testicular volume.

12) Fructose, Ph

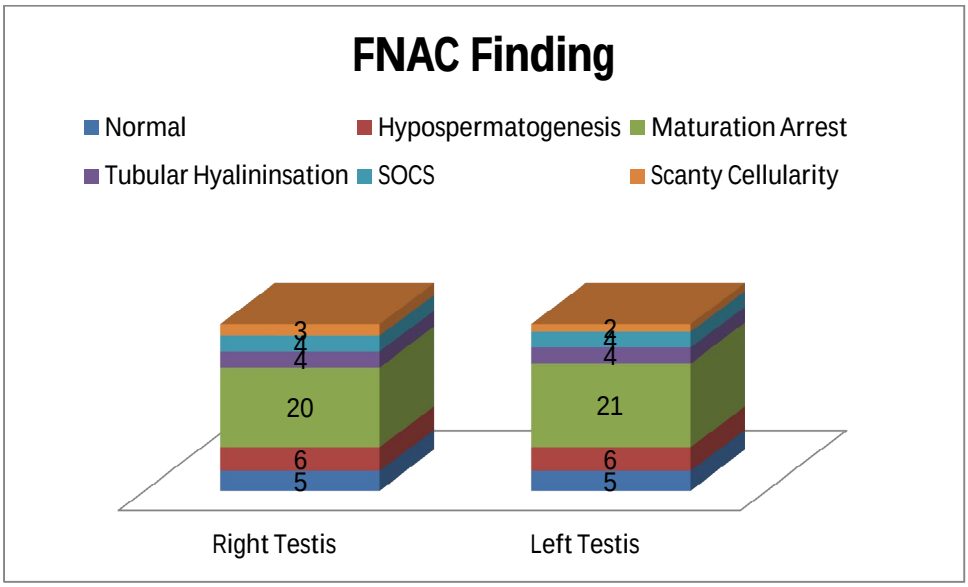
Fructose		ph	
Positive	**Negative**	**Acidic**	**Alkaline**
42 (100%)	0	0	42 (100%)



All the patients in the study had fructose present in their semen. The ph of semen was alkaline in all the patients included in the study.

13) FNAC Finding – Right and Left Testis

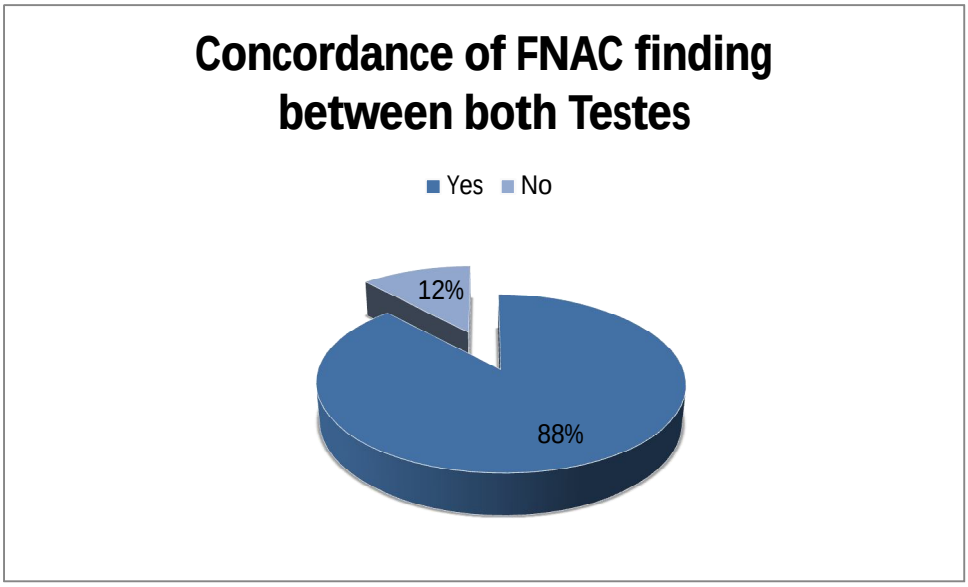
FNAC Finding	Right Testis (n = 42)	Left Testis (n =42)
Normal	5 (11.9%)	5 (11.9%)
Hypospermatogenesis	6 (14.3%)	6 (14.3%)
Maturation Arrest	20 (47.6%)	21 (50%)
Tubular Hyalininsation	4 (9.6%)	4 (9.6%)
SOCS	4 (9.6%)	4 (9.6%)
Scanty Cellularity	3 (7.2%)	2 (4.8%)



The most common finding in the Right testis FNAC group was maturation arrest (47.6%) followed by hypo spermatogenesis (14.3%) and normal spermatogenesis (11.9%). In the left testis FNAC group, the most common finding was again maturation arrest (50%) followed by hypo spermatogenesis (14.3%) and normal spermatogenesis (11.9%). The smear could not be reported in 3 patients (7.2%) of the right testis FNAC group and 2 patients (4.8%) of the left testis FNAC group because of scanty cellularity.

14) Concordance of FNAC finding between both Testes

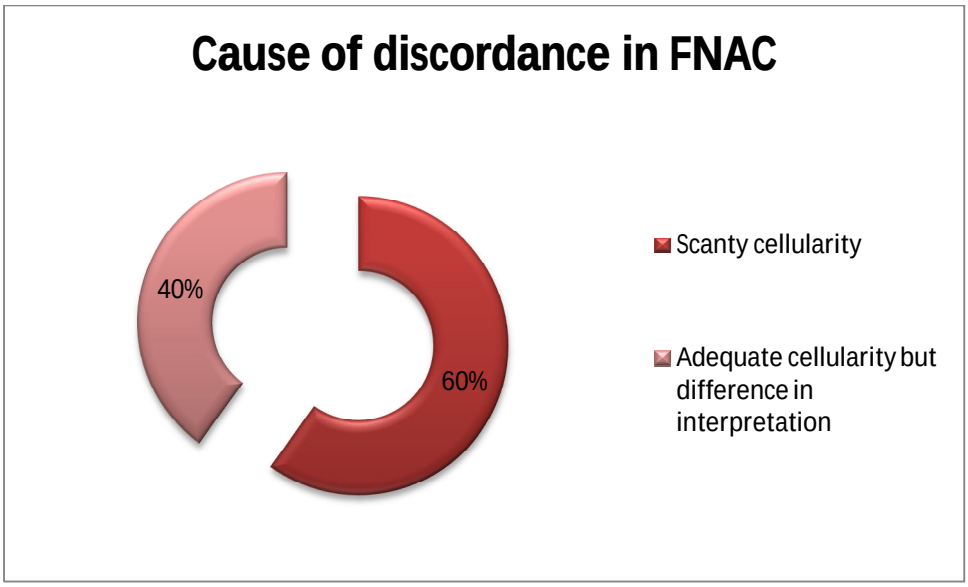
FNAC finding concordant	No. (n =42)
Yes	37 (88%)
No	5 (12%)



In our study, the results of FNAC between both the testes were comparable in 88% of the cases. They differed in 12 % of the cases.

15) Cause of Discordance

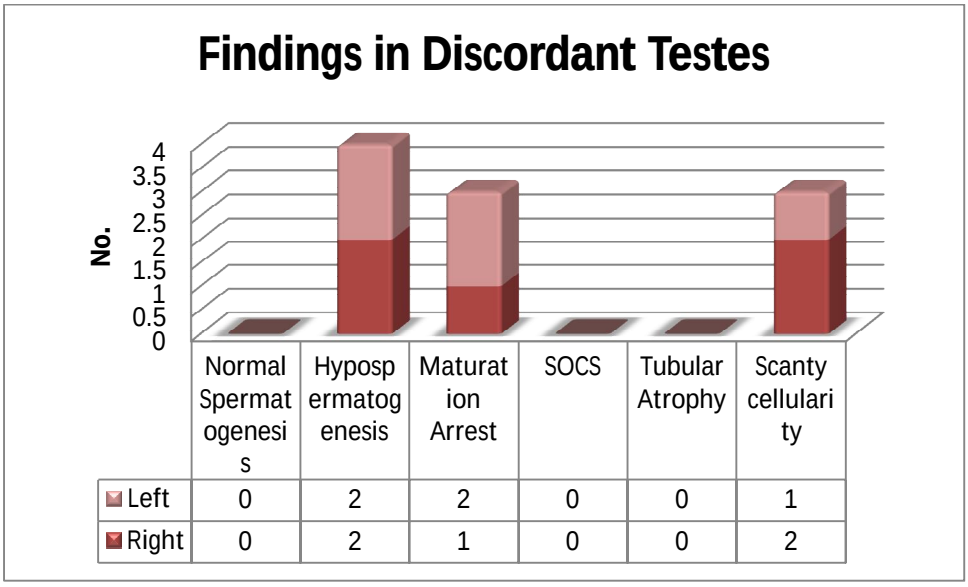
Cause of discordance in FNAC	No. (n =5)
Scanty cellularity	3 (60%)
Adequate cellularity but difference in interpretation	2 (40%)



Of the 5 cases in which there was discordance between the FNAC's results, in our study, 3 (60%) were due to difficulty in interpretation of the smear as a result of scanty cellularity.

16) Findings in Discordant Testes

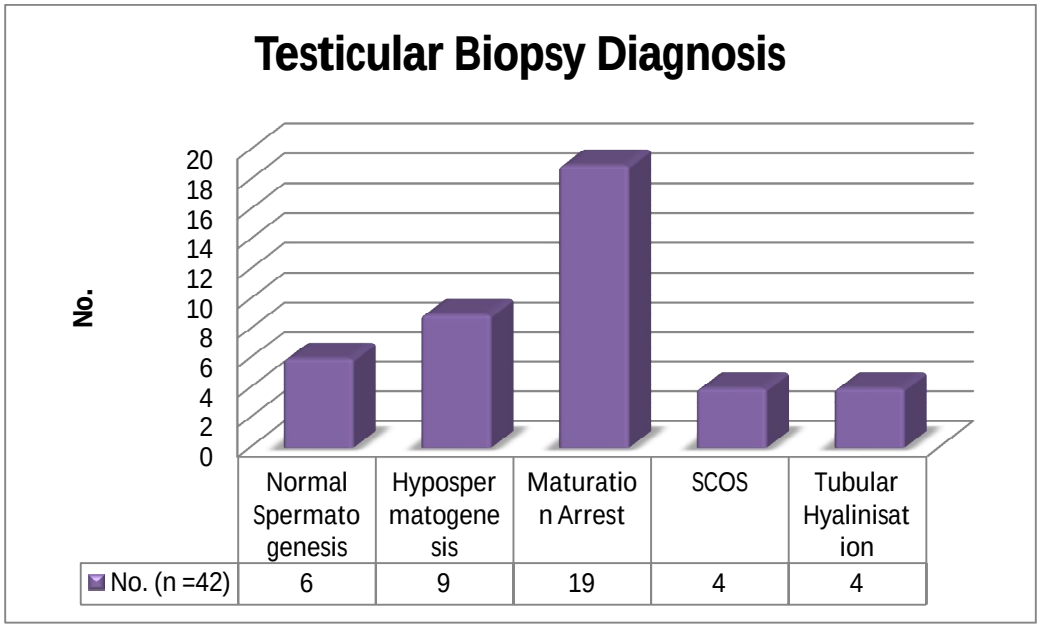
Finding in discordant Testes	Right (n =5)	Left (n =5)
Normal Spermatogenesis	0	0
Hypospermatogenesis	2 (40 %)	2 (40%)
Maturation Arrest	1 (20%)	2 (40%)
SOCS	0	0
Tubular Atrophy	0	0
Scanty cellularity	2 (40%)	1 (20%)



Of the 3 cases of scanty cellularity, 2 were noted on the right side and one on the left side. There was one case in which the smears from both the testes were inadequate for interpretation. Of the remaining two cases, in which, the smear was adequate and results were discordant, the difference of opinion was mainly between hypo spermatogenesis and maturation arrest.

17) Testicular Biopsy Diagnosis

Testicular Biopsy Findings	No. (n =42)
Normal Spermatogenesis	6 (14.3%)
Hypospermatogenesis	9 (21.4 %)
Maturation Arrest	19 (45.2%)
SCOS	4 (9.6%)
Tubular Hyalinisation	4 (9.6%)

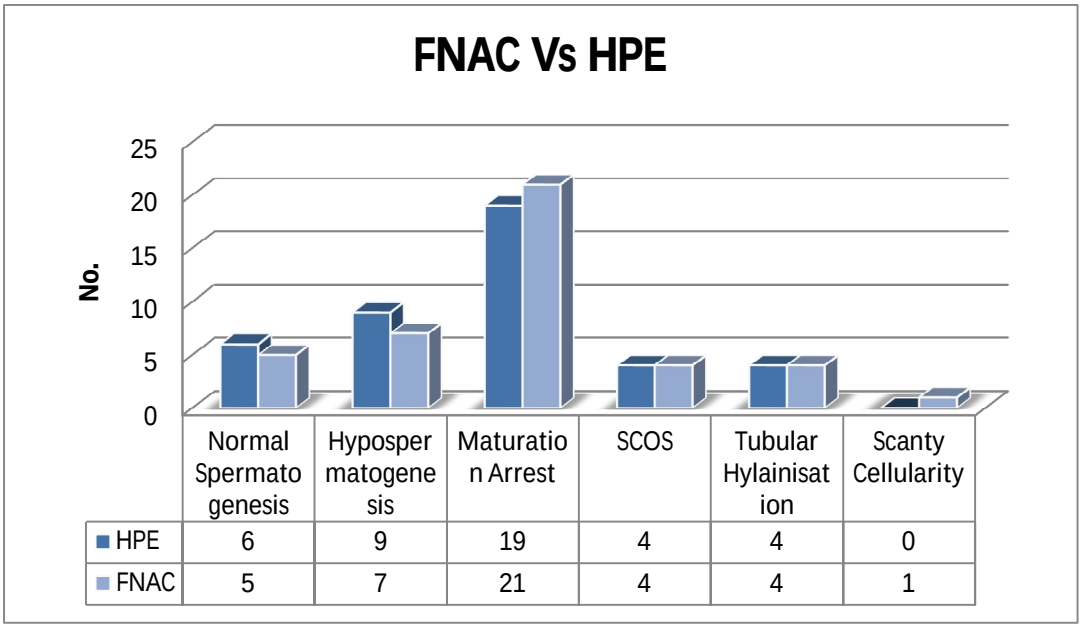


Maturation Arrest was the most common (45.2% of cases) diagnosis seen in testicular biopsy in our study. This was followed by hypo spermatogenesis (9 cases) and Normal spermatogenesis (6 cases).

There were 4 cases each of Sertoli Only Cell Syndrome (SCOS) and tubular hyalinisation. There were no cases in which the sample was found to be inadequate in contrast to FNAC, in our study.

18) FNAC – HPE Correlation

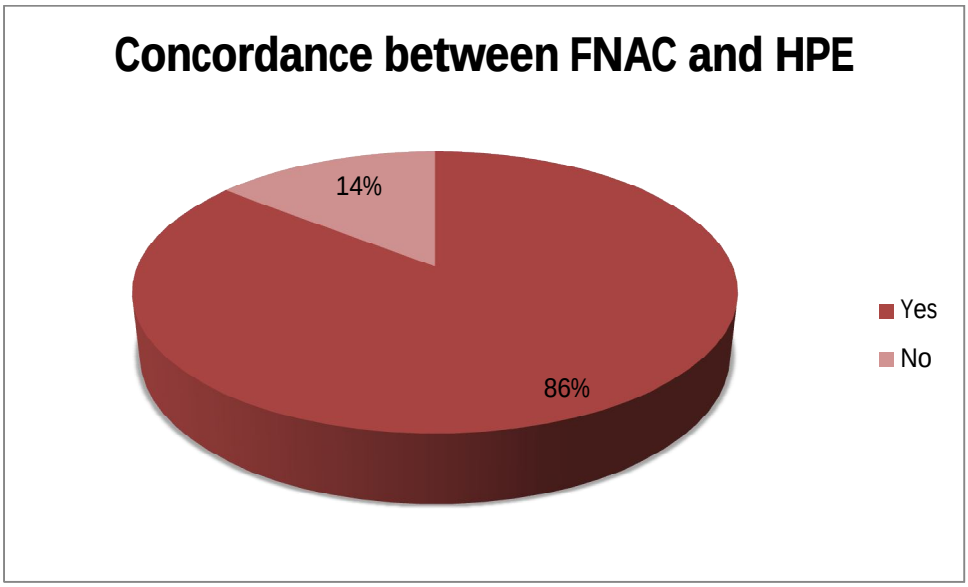
Finding	HPE (n =42)	FNAC (n =42)
Normal Spermatogenesis	6	5
Hypospermatogenesis	9	7
Maturation Arrest	19	21
SCOS	4	4
Tubular Hylainisation	4	4
Scanty Cellularity	0	1



Comparing the results of FNAC with testicular biopsy, in our study there was good concordance in cases with SCOS and tubular hyalinisation between the two (4 cases each). The most common diagnosis seen by both methods was Maturation arrest (45.2% with FNAC and 50 % with biopsy).

19) Concordance between FNAC and HPE

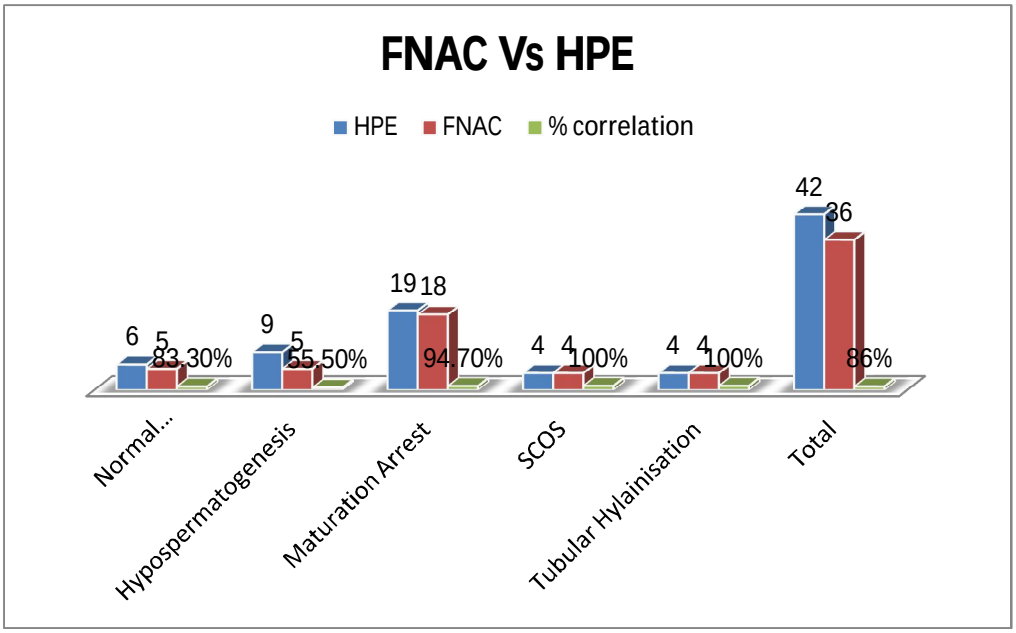
Concordance between FNAC and HPE	No.(n =42)
Yes	36 (86%)
No	6 (14 %)



The results of FNAC and HPE were comparable in 86% of the cases, in our study. There was difference of reports between FNAC and HPE in 14 % (6 cases) in our study.

20) Correlation between HPE and FNAC

Finding	HPE	FNAC	% correlation
Normal Spermatogenesis	6	5	83.30%
Hypospermatogenesis	9	5	55.50%
Maturation Arrest	19	18	94.70%
SCOS	4	4	100%
Tubular Hylainisation	4	4	100%
Total	**42**	**36**	**86%**



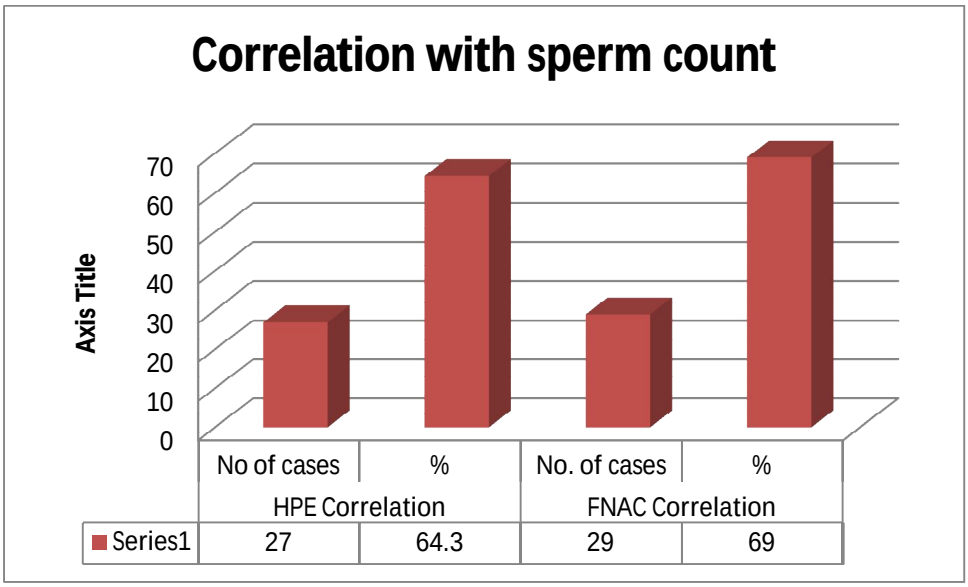
There was 100 % correlation between FNAC and HPE in patients with SCOS and Tubular Hyalinisation, in our study. Of the 6 cases reported as normal spermatogenesis by HPE, 5 cases were reported the same by FNAC (83.3% correlation).

The remaining one case was reported as hypo spermatogenesis due to presence of fewer mature spermatozoa in the smear. Of the 9 cases reported as hypo spermatogenesis by HPE, 5 cases reported the same by FNAC (55.5 % correlation).Among the remaining 4 cases, 3 were reported as maturation arrest and one was inadequate for opinion due to scanty

cellularity. Of the 19 cases of maturation arrest, there was good correlation with FNAC in 18 cases (94.7% correlation). One case was reported as hypo spermatogenesis.

21) Correlation between sperm count and HPE and cytological diagnosis

HPE Correlation		FNAC Correlation	
No of cases	**%**	**No. of cases**	**%**
27	64.3	29	69



In general, Azoospermia correlates with maturation arrest, germ cell aplasia and tubular hyalinization and Oligospermia correlates with hypospermatogenesis and normal spermatogenesis. In our study, out of the 42 cases of azoospermia, 27 cases (64.3%) correlated with HPE diagnosis and 29 cases (69%) correlated with FNAC.

22) FNAC – Sensitivity and Specificity

FNAC Finding	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Normal Spermatogenesis	83.30%	100%	100%	97.30%
Hypospermatogenesis	55.5.%	93.90%	71.40%	88.60%
Maturation Arrest	94.70%	87%	85 .7 %	95.20%
SCOS	100%	100%	100%	100%
Tubular Hyalinisation	100%	100%	100%	100%
Total	**86.70%**	**96.20%**	**91.40%**	**96.20%**

Considering testicular biopsy as the gold standard, in our study, FNAC was 100% sensitive and specific for the diagnosis of SCOS and tubular hyalinisation. With respect to normal spermatogenesis, FNAC was 83.3 %sensitive but 100 % specific and for hypo spermatogenesis it was 55.5 % sensitive and 93.9 % specific. In case of maturation arrest 94.7 % sensitive and 87% specific. This accounts for a overall sensitivity for FNAC of 86.7%, specificity of 96.2%, positive predictive value of 91.4 % and negative predictive value of 96.2%, in our study.

DISCUSSION

42 patients who fitted into the inclusion and exclusion criteria were enrolled in our study.

(i) *Age* :

The mean age in our study population was 31.83 years with a range of 25 to 43 years. This is comparable with other studies mentioned below.

Study	Mean age
R.C Adhikari et al (2009)[38]	30.9 years
Mallidis and Baker et al (1994)[39]	34 years
Present Study (2013)	31.83 years

(ii)*Duration and Type of Infertility* :

All the patients enrolled in the study were of primarily infertility type with a mean duration of 5.72 years .Majority (50%) of the patients presented 1- 4 years after marriage for evaluation of infertility. The results are comparable to the study done by Samal et al [40] in 2012. In this study the majority (32.9%) of the primary infertility patients presented for evaluation 1-4 years after marriage.

Duration of Infertility	Samal et al (2012)[40]	Present Study (2013)
1- 4 years	32.90%	50%
5-8 years	20%	33.30%
9-12 years	6.90%	9.50%
> 12 years	2.47%	4.70%

(iii) *History* :

In our study, history of mumps was present in 14%, while a history of inguinal/ scrotal surgery was present in 4.8%. The majority of our patients (71.4%) did not have any significant history at the time of presentation. These results are comparable to the study done by Khaled Madbouly et al in Saudi Arabia in 2012 [41]. The majority of their patients (94%) presented for evaluation of infertility alone without any antecedent positive history. History of mumps orchitis was present in 2% and history of inguinal/ scrotal surgery was present in 4 %. The incidence of mumps orchitis was lower in our study probably because of geographical factors and the increased number of patients (n=299) in the Saudi Arabian study.

History	Khaled Madbouly et al (2012) [41]	Present study (2013)
Nil significant	94%	71.40%
Mumps orchitis	2%	14%
Inguinal /scrotal surgery	4%	4.80%

(iv) *Testicular size and varicocele* :

Normal sized testes (> 18 cc by ultrasound) were present in 86% of our study population, while in the study by Khaled Madbouly et al [41] it was noted in 60% of the study population. 14 % of the study population in the present study had unilateral varicocele while 9.6% had bilateral varicocele.

In the study by Khaled Madbouly et al [41], similar results were noted (18 % had unilateral varicocele while 8% had bilateral varicocele).

Physical Examination	Khaled Madbouly et al (2012)[41]	Present study (2013)
Normal sized testes (> 18 cc)	60%	86.00%
Unilateral varicocele	18%	14.00%
Bilateral Varicocele	8%	9.60%

(v) *Hormone levels* :

The mean testosterone, FSH and LH values in the present study were 5.41 ng/ml, 7.94 mIU/L and 6.58 mIU/L respectively. These results are comparable again with the study done by Khaled Madbouly et al [41]. The incidence of hyperprolactinemia was low in both these studies. There were just 5 patients (2.2%) with elevated prolactin in the study by Khaled Madbouly et al [41], while in our study there were none.

Hormone Levels	Khaled Madbouly et al (2012) [41]	Present study (2013)
Testosterone (ng/ml)	7.2	5.41
FSH (mIU/L)	10.9	7.94
LH m(IU/L)	8.4	6.58

(vi) *FNAC – cytological types* :

The most common cytological finding in our study was Maturation arrest (49%). This is comparable with studies done by Plas et al [42] in 1999 and by Meng et al [44]in 2001.

Study	Normal Spermatogenesis	Hypospermatogenesis	Maturation Arrest	SCOS	Tubular Hylainisation
Plas et al (1999)[42]	14%	3%	36%	22%	7%
Meng et al (2001)[43]	13.80%	17.20%	33.30%	35.6%	0%
Present Study (2013)	11.90%	14.30%	49%	9.60%	9.60%

(vi) *Corellation between FNAC of Right and Left Testes* :

The concordance rate between the cytological findings of right and left testes was 88 % in our study. The main cause of discordance in our study was due to the presence of scanty cellularity in the smear.

Comparison of FNAC Right and Left testis

FNAC Lt Testis		Normal	Hypospermatogenisis	Maturation Arrest	Others	
FNAC Rt Testis	Normal	5	0	0	0	5
	Hypospermatogenisis	0	4	1	1	6
	Maturation Arrest	0	1	19	0	20
	Others	0	1	1	9	11
Total		5	6	21	10	42

(p value = 0.801) (Kappa= 0.821, p value <0.001)

The degree of difference between the two FNAC findings was not significant as shown by a p value of 0.801 as per Chi square test (Mc Nemar test). The degree of association between the two findings was however significant – kappa value of 0.821 ; p value of < 0.001.

These findings suggest that in a patient undergoing testicular FNAC for azoospermia, the findings between the right and left testes were comparable. Hence it may be more than sufficient to limit the FNAC to just one testis.

These results are comparable with that of the study done by Kurien A et al in 2003 [44]. In their study, the concordance rate between FNAC of right and left testes was 95 % (vs 88 % in the present study).

(vii) *Correlation between Testicular FNAC and Histopathology* :

The correlation between FNAC and Histopathology in our study was 86%.There were 6 cases in which there was a difference between FNAC and HPE findings in our study. Of the 4 cases reported as Hypo spermatogenesis by HPE, 3 were reported as Maturation arrest and one as Scanty Cellularity by FNAC. One case of Maturation Arrest was reported as Hypo spermatogenesis by FNAC and another case of Normal spermatogenesis was reported as Hypo spermatogenesis.

Comparison of Testicular Biopsy with FNAC of Lt Testis

Biopsy		Normal	Hypospermatogenisis	Maturation Arrest	Others	
FNAC Lt Testis	Normal	5	0	0	0	5
	Hypospermatogenisis	1	4	1	0	6
	Maturation Arrest	0	3	18	0	21
	Others	0	2	0	8	10
Total		6	9	19	8	42

(P value < 0.261 by Mc Nemar Test for the Biopsy and FNAC) (Kappa = 0.755, p < 0.00001 for concordance between two test)

Comparison of Testicular Biopsy with FNAC Rt Testis

Biopsy		Normal	Hypospermatogenisis	Maturation Arrest	Others	
FNAC Rt Testis	Normal	5	0	0	0	5
	Hypospermatogenisis	0	4	2	0	6
	Maturation Arrest	0	4	16	0	20
	Others	1	1	1	8	11
Total		6	9	19	8	42

(p value < 0.453, by Mc Nemar Test for the Biopsy and FNAC) (Kappa= 0.688, p < 0.00001 for concordance between two test)

The tables above show that there is good correlation between FNAC findings of both testes taken separately and the Histopathology reports. The difference between FNAC of right testis and HPE was not significant (p value of <0.453). The same was also noted for FNAC of the left testis (p value of < 0.261). These results are comparable with other studies shown below.

Study	No. of patients	Degree of correlation between FNAC and HPE
Craft et al (1997) [45]	19	84%
Rammou -Kinia et al (1999) [46]	30	87%
Meng et al (2001) [43]	87	94%
Qublan et al (2002) [47]	34	96%
Srivastava et al (2004) [48]	46	96%
Present Study (2013)	42	86%

The overall sensitivity and specificity in our study for FNAC was 86.7 % and 97.2 % respectively. The positive predictive value was 91.4% and negative predictive value was 96.2%. These results are comparable with other studies.

Study	Sensitivity	Specificity
Hussein et al(2005) [49]	98%	100%
Present study (2013)	86.70%	97.20%

(viii) Correlation between sperm count and HPE :

Present Study (2013)		Agarwal et al (2004) [50]	
No of cases	**%**	**No. of cases**	**%**
27	64.3	27	81

In general, azoospermia correlates with HPE diagnosis of maturation arrest, SCOS or tubular hylanisation. In our study, there was 74.3% correlation between HPE diagnosis and sperm count. This is comparable to a study done by Agarwal et al in 2004 [50].

FNAC of the testis is a simple, safe and effective outpatient procedure for the evaluation of azoospermia. It yields adequate material and in experienced hands, provides reliable diagnosis. An accurate diagnosis may be established by a combination of following parameters: clinical assessment of the patient, hormonal levels such as FSH and testosterone and use of testicular FNAC [1].

FIGURE -1
TESTICULAR BIOPSY

FIGURE -2
TESTICULAR BIOPSY

FIGURE -3
TESTICULAR FNAC

FIGURE -4
BOUIN'S SOLUTION, SLIDE, SYRINGE & NEEDLE

SUMMARY AND CONCLUSION

Testicular Fine Needle Aspiration Cytology is a reliable, quick, and easy technique that is also less invasive. It is associated with minimal or no complications. It is one of the investigations that can play an important role in diagnosis and management of infertility. FNAC also gives informative data on spermatogenesis of the entire testes. The use of Testicular FNAC has picked up in recent years following the works of Obrant et al ,Person et al, Papic et al, Schenck & Schill et al and Foresta et al [6]. They characterized the different cell types seen in cytological smears and demonstrated good correlation between these cytological categories and histological diagnosis. In the current era of microassisted fertilization techniques, which is of great help to the infertile couple, the only requirement is the availability of a viable sperm and ovum. Neither the quality nor the degree of motility is essential. Therefore in cytological smears, a report of presence or absence of sperm is adequate.

This study was done in the Department of Urology, Stanley Medical College and Hospital. 42 azoospermic males were included in the study. All the patients were of primary infertility. The mean age of the study population was 31.83 years and the mean duration of infertility was 5.72 years. The main aim of the study was to evaluate the cytological features of testicular FNAC in azoospermia, and to study the correlation between

cytological and histological diagnosis. The need for bilateral FNAC's was also evaluated.

Majority of the patients in our study did not have a positive history apart from the history of infertility. The clinical and hormonal evaluations were also with normal limits in the majority of the patients. These results were comparable with other similar studies.

Maturation arrest was the most common pattern seen in our study (45.2%) by histopathology. Next common was Hypospermatogenesis (21.4%) followed by Normal spermatogenesis (14.3%). The predominant Johnson's score ranged between 3 and 7 indicating maturation arrest at spermatogonia, spermatocyte and spermatid levels.

In FNAC, the most common diagnosis was again Maturation arrest (49%). Considering histopathology as the gold standard for correct diagnosis, the **correlation of FNAC with histopathology was 86%** (p value 0.801). Our study showed an overall sensitivity of 86.7%, specificity of 97.2%, positive predictive value of 91.4% and negative predictive value of 96.2%. No major complications were encountered in relation to the procedure.

Similarly there was also **good correlation (88%) between the FNAC reports of right and left testes**. This emphasises the fact that unilateral FNAC is more than sufficient for the evaluation of FNAC.

To conclude, FNAC of the testis can be considered as better than or equal to testicular biopsy in the evaluation of azoospermia for the following reasons:

i) It is simple, inexpensive and minimally traumatic with less number of complications.

ii) More sites can be aspirated safely. This is especially useful when the lesions are focal or mixed, in which conditions, histology can be misinterpreted.

iii) Cytomorphological features of various cell types can be identified correctlyand can evaluate accurately all classically defined histological types.

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PROFORMA

1) Name:
2) Age :
3) OP/ IP No:
4) Address and Phone No:

5) Duration of Infertility:
6) Primary or Secondary Infertility:
7) History:
   - H/O trauma, recurrent fever, mumps
   - H/O erectile dysfunction
   - H/O DM/HTN/PT
   - H/O prolonged drug intake/ alcohol/ smoking
   - Any other significant history

8) General Physical Examination :
   - Pallor/icterus/ cyanosis/ GLA
9) Systemic Examination :
   - CVS :
   - RS:
   - CNS:
   - P/A :

- Examination of Ext.Genitalia and Testes:

- DRE:

10) Investigations :

- CBC
- RBS,RFT
- Hormonal Assay
- Semen Analysis
    - Volume:
    - Alkaline / Acidic:
    - Liquefaction time:
    - Count:
    - Motility:
    - Morphology:
    - Fructose:
    - Additional features if any:
- USG /Duplex if done:

11) FNAC

- Date :
- No:
- Right testis:
- Left Testis:

- Final Impression:

12) Testicular Biopsy

- Date:
- No:
- Microscopic findings:
- Final Impression:

S.No	OP / IP no	Age	Duration of infertility	Primary /Secondary	History	Physical Examination	Routine Lab investigations	Hormone Assay				USG Scrotum		Semen Parameters						Testicular FNAC		Testicular Biopsy
								FSH (1.5 - 12.4 mIU /ml	LH (1.5 - 9.5 mIU /ml	Testosterone (2.8- 8.0 ng /ml)	PRL (4.04 - 15.2 ng /ml)	Rt Testis	Lt Testis	Volume	Count (millions /ml)	Ph	Motility (a + b)	Morphology	Fructose	Right Testis	Left Testis	
1	3178/11	35 yrs	5yrs	Primary	Nil significant	Nil significant	WNL	18.69	4.7	2.2	13.6	18.3 cc. No varicocele	22.62 cc. No varicocele	1.2ml	Azoospermia	Alkaline	Nil	Nil	Positive	Hypospermatogenesis	Hypospermatogenesis	Hypospermatogensis.Johnson score : 8/10
2	2045/11	29yrs	5 yrs	Primary	H/O typhoid fever and Chicken pox @ age of 10 & 18yrs resp.	Left Testis smaller with Gr I varicocele	WNL	32.76	11.83	6.39	9.8	20.3 cc. No varicocele	16.5 cc. Gr I varicocele	3ml	Azoospermia	Alkaline	Nil	Nil	Positive	Hypospermatogenesis	Scanty cellularity	Hypospermatogensis.Johnson score : 8/10
3	4339/11	31yrs	4yrs	Primary	Nil significant	Nil significant	WNL	8.4	6.8	7.3	10.9	22.3cc. No varicocele	23.8cc. No varicocele	1.0 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 7/10
4	4387/11	32yrs	8yrs	Primary	Nil significant	Nil significant	WNL	10.3	7.4	6.6	9.5	21.1cc. No varicocele	23.4cc. No varicocele	1.2ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 5/10
5	4389/11	37yrs	7yrs	Primary	Nil significant	Nil significant	WNL	2.97	3.51	4.08	8.15	18.1cc. No varicocele	20.8cc. No varicocele	2.0ml	Azoospermia	Alkaline	Nil	Nil	Positive	Normal Spermatogenesis	Normal Spermatogenesis	Normal Spermatogenesis. Johnson Score 9/10
6	4532/11	35yrs	6yrs	Primary	H/O mumps at the age of 10yrs	B/L small testis with Gr I varicocele on the Right & Gr II on the Left	WNL	9.4	10.6	3.1	7.8	14.3cc .Gr III varcicocle	15.8cc .Gr IV varcicocle	2 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Scanty cellularity	Scanty cellularity	Hypospermatogensis.Johnson score : 8/10
7	4533/11	30yrs	6yrs	Primary	Nil significant	B/L Normal Testis with Left Gr I Varicocele	WNL	4.9	3.7	5.6	7.3	20.8cc. No varicocele	22.4cc .Gr I varicocele	1.5 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 6/10
8	4709/11	29yrs	4yrs	Primary	H/O mumps at the age of 9yrs	Nil significant	WNL	8.48	7.37	4.34	5.2	19.6cc. No varicocele	22.9cc .Gr I varicocele	4ml	Azoospermia	Alkaline	Nil	Nil	Positive	Hypospermatogensis	Hypospermatogensis	Maturation Arrest. Johnson Score 7/10
9	2648/09	38yrs	7yrs	Primary	H/O mumps at the age of 10yrs; H/O hydrocelectomy at the age of 23yrs .	B/L small testis with Gr II varicocele on the Right & Gr III on the Left	WNL	9.4	8.2	3.44	10.8	16.3cc .Gr III varcicocle	15.8cc .Gr IV varcicocle	0.8ml	Azoospermia	Alkaline	Nil	Nil	Positive	Sertoli only cell Syndrome	Sertoli only cell Syndrome	Sertoli only cell Syndrome; Johnson score 2/10
10	5033/11	28yrs	4yrs	Primary	Nil significant	Nil significant	WNL	5.64	6.78	6.6	7.32	23.8cc . No varicocele	24.4cc .Gr I varicocele	4.2ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 7/10
11	5071/11	31yrs	2yrs	Primary	Nil significant	Nil significant	WNL	8.2	7.4	5.92	4.89	21.5cc . No varicocele	23.7cc . No varicocele	3.8ml	Azoospermia	Alkaline	Nil	Nil	Positive	Hypospermatogensis	Maturation Arrest	Maturation Arrest. Johnson Score 6/10
12	1102/11	36yrs	5yrs	Primary	Nil significant	Nil significant	WNL	7.1	6.9	6.2	5.4	22.1cc . No varicocele	23.4cc . No varicocele	1 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 7/10
13	6115/11	34yrs	9yrs	Primary	Nil significant	B/L small testis ;No varicocele	WNL	12.93	9.1	4.2	9.6	13.7cc . No varicocele	15.4cc . No varicocele	1ml	Azoospermia	Alkaline	Nil	Nil	Positive	Tubular Hyalinisation	Tubular Hyalinisation	Tubular Hyalinisation; Johnson score 1/10
14	141/12	29yrs	4yrs	Primary	Nil significant	Nil significant	WNL	5.8	6.3	6.9	7.2	21.2cc . No varicocele	22.7cc . No varicocele	1.2ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 7/10
15	231/12	27 yrs	2 yrs	Primary	Nil significant	Nil significant	WNL	10.23	7.12	5.96	8.1	20.5 cc. No varicocele	19.4 cc .Gr I varicocele	1ml	Azoospermia	Alkaline	Nil	Nil	Positive	Tubular Hyalinisation	Tubular Hyalinisation	Tubular Hyalinisation; Johnson score 1/10
16	6362/11	26yrs	1.5yrs	Primary	Nil significant	B/L normal sized testes with B/L Gr Ivaricocele and Left indirect inguinal hernia	RBS : 250. Rest WNL	5.37	6.2	5.8	9.7	19.9 cc. Gr I varicocele	21.2 cc .Gr I varicocele	4.5ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 7/10
17	141/12	26 yrs	2yrs	Primary	Nil significant	Nil significant	WNL	7.2	8.3	5.8	6.3	22.1cc . No varicocele	23.9cc . No varicocele	2.5ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 6/10
18	244/12	31 yrs	3yrs	Primary	B/L inguinal hernia repair done 8yrs back	B/L inguinal scar present;B/L testes normal ; Left Gr II varicocele present	WNL	5.9	7.1	8.5	6.7	20.7cc . No varicocele	21.9 cc. Gr II varicocele	2ml	Azoospermia	Alkaline	Nil	Nil	Positive	Normal Spermatogenesis	Normal Spermatogenesis	Normal Spermatogenesis. Johnson Score 9/10
19	298/12	35yrs	7yrs	Primary	H/O mumps at the age of 11yrs	B/L Normal Testis with Right Gr II and Left Gr I Varicocele	WNL	6.1	6.8	7.2	6.6	18.9 cc. Gr II varicocele	20.5 cc .Gr I varicocele	1ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Hypospermatogensis.Johnson score : 8/10
20	683/11	27yrs	3yrs	Primary	Nil significant	Nil significant	WNL	5.8	4.9	3.4	12.4	20.2cc . No varicocele	20.5cc . No varicocele	1ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 7/10
21	332/12	39yrs	11 yrs	Primary	H/O mumps at the age of 10yrs ; Taking mmedications for Depression	B/L Hypoplastic testes with Gr I varicocoele on the Left side	WNL	15.3	8.4	3.9	10.6	10.2cc . No varicocele	10.8cc . Gr I varicocele	1ml	Azoospermia	Alkaline	Nil	Nil	Positive	Tubular Hyalinisation	Tubular Hyalinisation	Tubular Hyalinisation; Johnson score 1/10
22	244/12	31 yrs	3 yrs	Primary	B/L inguinal hernia repair done 9yrs back	B/L Normal Testis with B/L Gr II Varicocele	WNL	6.7	7.3	5.9	5.7	20.1 cc. Gr II varicocele	20.8 cc. Gr II varicocele	1ml	Azoospermia	Alkaline	Nil	Nil	Positive	Normal Spermatogenesis	Normal Spermatogenesis	Normal Spermatogenesis. Johnson Score 9/10
23	1848/11	31yrs	2 yrs	Primary	Nil significant	Nil significant	WNL	7.2	8.1	8.3	7.6	21.8cc . No varicocele	22.1cc . No varicocele	3 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Normal Spermatogenesis	Normal Spermatogenesis	Normal Spermatogenesis. Johnson Score 9/10
24	774/12	33 yrs	12 yrs	Primary	H/O mumps at the age of 8 yrs	Nil significant	WNL	6.81	4.41	5.43	8.2	19.9cc . No varicocele	20.2cc . No varicocele	3ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 7/10
25	912/12	43 yrs	15 yrs	Primary	F/S/O Klinefelter's syndrome	B/L nubbin testes	RBS 201	18.3	16.8	1.1	12.3	4.2cc. No varcicocele	4.5 cc No varcicocele	0.5ml	Azoospermia	Alkaline	Nil	Nil	Positive	Tubular Hyalinisation	Tubular Hyalinisation	Tubular Hyalinisation; Johnson score 1/10
26	1077/12	28 yrs	2 yrs	Primary	Nil significant	Nil significant	WNL	3.2	3.9	8.4	6.9	20.8cc . No varicocele	21.5cc . No varicocele	2.5 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Hypospermatogensis.Johnson score : 8/10
27	1096 / 12	29 yrs	2 yrs	Primary	Nil significant	Nil significant	WNL	4.1	6.2	4.28	8.12	21.7cc . No varicocele	22.3cc . No varicocele	1 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 6/10
28	1446/ 12	25 yrs	3 yrs	Primary	Nil significant	Nil significant	WNL	5.12	6.43	4.45	5.89	19.6cc . No varicocele	20.5cc . No varicocele	3 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 6/10
29	1448/12	38yrs	14yrs	Primary	Nil significant	Nil significant	WNL	6.36	4.12	7.82	4.87	20.3cc . No varicocele	20.7cc . No varicocele	1.5ml	Azoospermia	Alkaline	Nil	Nil	Positive	Hypospermatogensis	Hypospermatogensis	Hypospermatogensis.Johnson score : 8/10
30	1548/12	35 yrs	8 yrs	Primary	Nil significant	Nil significant	WNL	3.76	4.28	4.77	5.1	19.5cc . No varicocele	18.9cc . No varicocele	1 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Sertoli only cell Syndrome	Sertoli only cell Syndrome	Sertoli only cell Syndrome; Johnson score 2/10
31	1911/12	30 yrs	3 yrs	Primary	Nil significant	Nil significant	WNL	5.98	4.45	6.29	7.11	20.1cc . No varicocele	20.6cc . No varicocele	1.5 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Normal Spermatogenesis	Normal Spermatogenesis	Normal Spermatogenesis. Johnson Score 9/10
32	1984/12	42 yrs	20yrs	Primary	Nil significant	Nil significant	WNL	3.7	3.2	5.4	6.9	19.2cc . No varicocele	19.5cc . No varicocele	1ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 5/10
33	2870/12	25 yrs	2 yrs	Primary	Nil significant	Nil significant	WNL	7.1	5.2	6.33	6.1	18.4cc . No varicocele	19.8cc . No varicocele	1ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Hypospermatogensis.Johnson score : 8/10
34	2858/12	31 yrs	5 yrs	Primary	Nil significant	Nil significant	WNL	6.2	5.8	4.12	5.6	20.1cc . No varicocele	20.8cc . GR I varicocele	2ml	Azoospermia	Alkaline	Nil	Nil	Positive	Scanty cellularity	Maturation Arrest	Maturation Arrest. Johnson Score 7/10
35	5923/11	31 yrs	1 yr	Primary	H/O portal HTN present ; Splenectomy with devascularisation done 10yrs back	Nil significant apart from abdominal scar which was healthy	WNL	8.12	7.22	3.1	6.34	18.3cc . No varicocele	19.1cc . No varicocele	2 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Sertoli only cell Syndrome	Sertoli only cell Syndrome	Sertoli only cell Syndrome; Johnson score 2/10
36	221/12	26 yrs	2.5 yrs	Primary	Nil significant	Nil significant	WNL	5.11	8.22	6.3	5.6	20.5cc . No varicocele	21.1cc . No varicocele	2.5 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Hypospermatogensis	Hypospermatogensis	Hypospermatogensis.Johnson score : 8/10
37	3286/12	41 yrs	4 yrs	Primary	Nil significant	B/L Normal Testis with B/L Gr I Varicocele	WNL	8.62	7.3	2.94	8.74	20.8cc . GR I varicocele	21.0 cc . GR I varicocele	0.5ml	Azoospermia	Alkaline	Nil	Nil	Positive	Scanty cellularity	Hypospermatogensis	Normal Spermatogenesis. Johnson Score 9/10
38	3564/12	28 yrs	7 yrs	Primary	Nil significant	Nil significant	WNL	6.03	6.89	4.33	7.93	19.9cc . GR I varicocele	21.1 cc . GR I varicocele	1.5ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 6/10
39	399/12	35 yrs	5yrs	Primary	Nil significant	Nil significant	WNL	5.22	4.16	6.94	4.99	18.7cc . No varicocele	19.6cc . No varicocele	2 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Sertoli only cell Syndrome	Sertoli only cell Syndrome	Sertoli only cell Syndrome; Johnson score 2/10
40	3883/12	40 yrs	12 yrs	Primary	DM x 3yrs	Nil significant	WNL	4.19	5.99	4.52	6.24	21.1cc . No varicocele	21.5cc . No varicocele	1 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 6/10
41	3419/12	28 yrs	8 yrs	Primary	Nil significant	Nil significant	WNL	6.05	4.12	7.11	5.52	21.2cc . No varicocele	21.9cc . No varicocele	2ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Hypospermatogensis	Hypospermatogensis.Johnson score : 8/10
42	3797/12	26 yrs	3 yrs	Primary	Nil significant	Nil significant	WNL	4.8	3.23	5.65	6.24	20.5cc . No varicocele	21.1cc . No varicocele	3.5 ml	Azoospermia	Alkaline	Nil	Nil	Positive	Maturation Arrest	Maturation Arrest	Maturation Arrest. Johnson Score 7/10